

Title: Inbred Maize Line PHCWK 5	Anticipated Class: 800 Subclass: 200 Art Unit: 1638
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## FIELD OF THE INVENTION

This invention relates generally to the field of maize breeding, specifically  
10 relating to an inbred maize line designated PHCWK.

## BACKGROUND OF THE INVENTION

The goal of plant breeding is to combine, in a single variety or hybrid, various  
desirable traits. For field crops, these traits may include resistance to diseases and  
15 insects, tolerance to heat and drought, reducing the time to crop maturity, greater  
yield, and better agronomic quality. With mechanical harvesting of many crops,  
uniformity of plant characteristics such as germination, stand establishment, growth  
rate, maturity, plant height and ear height, is important. Traditional plant breeding is  
an important tool in developing new and improved commercial crops.  
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## SUMMARY OF THE INVENTION

According to the invention, there is provided a novel inbred maize line,  
designated PHCWK and processes for making PHCWK. This invention relates to  
seed of inbred maize line PHCWK, to the plants of inbred maize line PHCWK, to  
25 plant parts of inbred maize line PHCWK, and to processes for making a maize plant  
that comprise crossing inbred maize line PHCWK with another maize plant. This  
invention also relates to processes for making a maize plant containing in its genetic  
material one or more traits introgressed into PHCWK through backcross conversion  
and/or transformation, and to the maize seed, plant and plant part produced by such  
30 introduction. This invention further relates to a hybrid maize seed, plant or plant  
part produced by crossing the inbred line PHCWK or an introgressed trait conversion  
of PHCWK with another maize line. This invention also relates to inbred maize lines  
derived from inbred maize line PHCWK, to processes for making other inbred maize

lines derived from inbred maize line PHCWK and to the inbred maize lines and their parts derived by the use of those processes.

## Definitions

5        Certain definitions used in the specification are provided below. Also in the examples that follow, a number of terms are used herein. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided. NOTE: ABS is in absolute terms and %MN is percent of the mean for the experiments in which the  
10      inbred or hybrid was grown. PCT designates that the trait is calculated as a percentage. %NOT designates the percentage of plants that did not exhibit a trait. For example, STKLDG %NOT is the percentage of plants in a plot that were not stalk lodged. These designators will follow the descriptors to denote how the values are to be interpreted.

15      ABTSTK = ARTIFICIAL BRITTLE STALK. A count of the number of “snapped” plants per plot following machine snapping. A snapped plant has its stalk completely snapped at a node between the base of the plant and the node above the ear. Expressed as percent of plants that did not snap.

ALLELE. Any of one or more alternative forms of a genetic sequence.

20      Typically, in a diploid cell or organism, the two alleles of a given sequence typically occupy corresponding loci on a pair of homologous chromosomes.

ANT ROT = ANTHRACNOSE STALK ROT (*Colletotrichum graminicola*). A 1 to 9 visual rating indicating the resistance to Anthracnose Stalk Rot. A higher score indicates a higher resistance.

25      BACKCROSSING. Process in which a breeder crosses a hybrid progeny line back to one of the parental genotypes one or more times.

BARPLT = BARREN PLANTS. The percent of plants per plot that were not barren (lack ears).

BREEDING. The genetic manipulation of living organisms.

30      BREEDING CROSS. A cross to introduce new genetic material into a plant for the development of a new variety. For example, one could cross plant A with plant B,

wherein plant B would be genetically different from plant A. After the breeding cross, the resulting F1 plants could then be selfed or sibbed for one, two, three or more times (F1, F2, F3, etc.) until a new inbred variety is developed. For clarification, such new inbred varieties would be within a pedigree distance of one breeding cross of 5 plants A and B. The process described above would be referred to as one breeding cycle.

BRTSTK = BRITTLE STALKS. This is a measure of the stalk breakage near the time of pollination, and is an indication of whether a hybrid or inbred would snap or break near the time of flowering under severe winds. Data are presented as 10 percentage of plants that did not snap.

CELL. Cell as used herein includes a plant cell, whether isolated, in tissue culture or incorporated in a plant or plant part.

CLDTST = COLD TEST. The percent of plants that germinate under cold test conditions.

15 CLN = CORN LETHAL NECROSIS. Synergistic interaction of maize chlorotic mottle virus (MCMV) in combination with either maize dwarf mosaic virus (MDMV-A or MDMV-B) or wheat streak mosaic virus (WSMV). A 1 to 9 visual rating indicating the resistance to Corn Lethal Necrosis. A higher score indicates a higher resistance.

20 COMRST = COMMON RUST (*Puccinia sorghi*). A 1 to 9 visual rating indicating the resistance to Common Rust. A higher score indicates a higher resistance.

CROSS POLLINATION. A plant is cross pollinated if the pollen comes from a flower on a different plant from a different family or line. Cross pollination excludes sib and self pollination.

25 CROSS. As used herein, the term "cross" or "crossing" can refer to a simple X by Y cross, or the process of backcrossing, depending on the context.

D/D = DRYDOWN. This represents the relative rate at which a hybrid will reach acceptable harvest moisture compared to other hybrids on a 1 to 9 rating scale. A high score indicates a hybrid that dries relatively fast while a low score 30 indicates a hybrid that dries slowly.

DIPERS = DIPLODIA EAR MOLD SCORES (*Diplodia maydis* and *Diplodia macrospora*). A 1 to 9 visual rating indicating the resistance to Diplodia Ear Mold. A higher score indicates a higher resistance.

5 DIPLOID PLANT PART. Refers to a plant part or cell that has the same diploid genotype as PHCWK.

DIPROT = DIPLODIA STALK ROT SCORE. Score of stalk rot severity due to *Diplodia* (*Diplodia maydis*). Expressed as a 1 to 9 score with 9 being highly resistant.

DRPEAR = DROPPED EARS. A measure of the number of dropped ears per plot and represents the percentage of plants that did not drop ears prior to harvest.

10 D/T = DROUGHT TOLERANCE. This represents a 1 to 9 rating for drought tolerance, and is based on data obtained under stress conditions. A high score indicates good drought tolerance and a low score indicates poor drought tolerance.

EARHT = EAR HEIGHT. The ear height is a measure from the ground to the highest placed developed ear node attachment and is measured in centimeters.

15 EARMED = GENERAL EAR MOLD. Visual rating (1 to 9 score) where a "1" is very susceptible and a "9" is very resistant. This is based on overall rating for ear mold of mature ears without determining the specific mold organism, and may not be predictive for a specific ear mold.

20 EARSZ = EAR SIZE. A 1 to 9 visual rating of ear size. The higher the rating the larger the ear size.

EBTSTK = EARLY BRITTLE STALK. A count of the number of "snapped" plants per plot following severe winds when the corn plant is experiencing very rapid vegetative growth in the V5-V8 stage. Expressed as percent of plants that did not snap.

25 ECB1LF = EUROPEAN CORN BORER FIRST GENERATION LEAF FEEDING (*Ostrinia nubilalis*). A 1 to 9 visual rating indicating the resistance to preflowering leaf feeding by first generation European Corn Borer. A higher score indicates a higher resistance.

ECB2IT = EUROPEAN CORN BORER SECOND GENERATION INCHES OF  
30 TUNNELING (*Ostrinia nubilalis*). Average inches of tunneling per plant in the stalk.

ECB2SC = EUROPEAN CORN BORER SECOND GENERATION (*Ostrinia nubilalis*). A 1 to 9 visual rating indicating post flowering degree of stalk breakage and other evidence of feeding by European Corn Borer, Second Generation. A higher score indicates a higher resistance.

5 ECBDPE = EUROPEAN CORN BORER DROPPED EARS (*Ostrinia nubilalis*). Dropped ears due to European Corn Borer. Percentage of plants that did not drop ears under second generation corn borer infestation.

10 EGRWTH = EARLY GROWTH. This is a measure of the relative height and size of a corn seedling at the 2-4 leaf stage of growth. This is a visual rating (1 to 9), with 1 being weak or slow growth, 5 being average growth and 9 being strong growth. Taller plants, wider leaves, more green mass and darker color constitute higher score.

15 ELITE INBRED. An inbred that contributed desirable qualities when used to produce commercial hybrids. An elite inbred may also be used in further breeding for the purpose of developing further improved varieties.

ERTLDG = EARLY ROOT LODGING. Early root lodging is the percentage of plants that do not root lodge prior to or around anthesis; plants that lean from the vertical axis at an approximately 30 degree angle or greater would be counted as root lodged.

20 ERTLDPN = EARLY ROOT LODGING. An estimate of the percentage of plants that do not root lodge prior to or around anthesis; plants that lean from the vertical axis at an approximately 30 degree angle or greater would be considered as root lodged.

25 ERTLSC = EARLY ROOT LODGING SCORE. Score for severity of plants that lean from a vertical axis at an approximate 30 degree angle or greater which typically results from strong winds prior to or around flowering recorded within 2 weeks of a wind event. Expressed as a 1 to 9 score with 9 being no lodging.

30 ESTCNT = EARLY STAND COUNT. This is a measure of the stand establishment in the spring and represents the number of plants that emerge on per plot basis for the inbred or hybrid.

EYESPT = EYE SPOT (*Kabatiella zeae* or *Aureobasidium zeae*). A 1 to 9 visual rating indicating the resistance to Eye Spot. A higher score indicates a higher resistance.

FUSERS = FUSARIUM EAR ROT SCORE (*Fusarium moniliforme* or *Fusarium subglutinans*). A 1 to 9 visual rating indicating the resistance to Fusarium ear rot. A higher score indicates a higher resistance.

GDU = GROWING DEGREE UNITS. Using the Barger Heat Unit Theory, which assumes that maize growth occurs in the temperature range 50 degrees F – 86 degrees F and that temperatures outside this range slow down growth; the maximum daily heat unit accumulation is 36 and the minimum daily heat unit accumulation is 0. The seasonal accumulation of GDU is a major factor in determining maturity zones.

GDUSHD = GDU TO SHED. The number of growing degree units (GDUs) or heat units required for an inbred line or hybrid to have approximately 50 percent of the plants shedding pollen and is measured from the time of planting. Growing degree units are calculated by the Barger Method, where the heat units for a 24-hour period are:

$$\text{GDU} = \frac{(\text{Max. temp.} + \text{Min. temp.}) - 50}{2}$$

The highest maximum temperature used is 86 degrees F. and the lowest minimum temperature used is 50 degrees F. For each inbred or hybrid it takes a certain number of GDUs to reach various stages of plant development.

GDUSLK = GDU TO SILK. The number of growing degree units required for an inbred line or hybrid to have approximately 50 percent of the plants with silk emergence from time of planting. Growing degree units are calculated by the Barger Method as given in GDU SHD definition.

GENOTYPE. Refers to the genetic constitution of a cell or organism.

GIBERS = GIBBERELLA EAR ROT (PINK MOLD) (*Gibberella zeae*). A 1 to 9 visual rating indicating the resistance to Gibberella Ear Rot. A higher score indicates a higher resistance.

GIBROT = GIBBERELLA STALK ROT SCORE. Score of stalk rot severity due to Gibberella (*Gibberella zeae*). Expressed as a 1 to 9 score with 9 being highly resistant.

5 GLFSPT = GRAY LEAF SPOT (*Cercospora zeae-maydis*). A 1 to 9 visual rating indicating the resistance to Gray Leaf Spot. A higher score indicates a higher resistance.

GOSWLT = GOSS' WILT (*Corynebacterium nebrascense*). A 1 to 9 visual rating indicating the resistance to Goss' Wilt. A higher score indicates a higher resistance.

10 GRNAPP = GRAIN APPEARANCE. This is a 1 to 9 rating for the general appearance of the shelled grain as it is harvested based on such factors as the color of harvested grain, any mold on the grain, and any cracked grain. High scores indicate good grain visual quality.

15 HAPLOID PLANT PART. Refers to a plant part or cell that has the same haploid genotype as PHCWK.

HCBLT = HELMINTHOSPORIUM CARBONUM LEAF BLIGHT (*Helminthosporium carbonum*). A 1 to 9 visual rating indicating the resistance to Helminthosporium infection. A higher score indicates a higher resistance.

20 HD SMT = HEAD SMUT (*Sphacelotheca reiliana*). This score indicates the percentage of plants not infected.

25 HSKCVR = HUSK COVER. A 1 to 9 score based on performance relative to key checks, with a score of 1 indicating very short husks, tip of ear and kernels showing; 5 is intermediate coverage of the ear under most conditions, sometimes with thin husk; and a 9 has husks extending and closed beyond the tip of the ear. Scoring can best be done near physiological maturity stage or any time during dry down until harvested.

INC D/A = GROSS INCOME (DOLLARS PER ACRE). Relative income per acre assuming drying costs of two cents per point above 15.5 percent harvest moisture and current market price per bushel.

30 INCOME/ACRE. Income advantage of hybrid to be patented over other hybrid on per acre basis.

INC ADV = GROSS INCOME ADVANTAGE. GROSS INCOME advantage of variety #1 over variety #2.

KSZDCD = KERNEL SIZE DISCARD. The percent of discard seed; calculated as the sum of discarded tip kernels and extra large kernels.

5 LINKAGE. Refers to a phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.

LINKAGE DISEQUILIBRIUM. Refers to a phenomenon wherein alleles tend to remain together in linkage groups when segregating from parents to offspring, with  
10 a greater frequency than expected from their individual frequencies.

L/POP = YIELD AT LOW DENSITY. Yield ability at relatively low plant densities on a 1 to 9 relative system with a higher number indicating the hybrid responds well to low plant densities for yield relative to other hybrids. A 1, 5, and 9 would represent very poor, average, and very good yield response, respectively, to  
15 low plant density.

LRTLDG = LATE ROOT LODGING. Late root lodging is the percentage of plants that do not root lodge after anthesis through harvest; plants that lean from the vertical axis at an approximately 30 degree angle or greater would be counted as root lodged.

20 LRTLDPN = LATE ROOT LODGING. Late root lodging is an estimate of the percentage of plants that do not root lodge after anthesis through harvest; plants that lean from the vertical axis at an approximately 30 degree angle or greater would be considered as root lodged.

25 LRTLSC = LATE ROOT LODGING SCORE. Score for severity of plants that lean from a vertical axis at an approximate 30 degree angle or greater which typically results from strong winds after flowering. Recorded prior to harvest when a root-lodging event has occurred. This lodging results in plants that are leaned or "lodged" over at the base of the plant and do not straighten or "goose-neck" back to a vertical position. Expressed as a 1 to 9 score with 9 being no lodging.

30 MDMCPX = MAIZE DWARF MOSAIC COMPLEX (MDMV = Maize Dwarf Mosaic Virus and MCDV = Maize Chlorotic Dwarf Virus). A 1 to 9 visual rating

indicating the resistance to Maize Dwarf Mosaic Complex. A higher score indicates a higher resistance.

MST = HARVEST MOISTURE. The moisture is the actual percentage moisture of the grain at harvest.

5 MSTADV = MOISTURE ADVANTAGE. The moisture advantage of variety #1 over variety #2 as calculated by: MOISTURE of variety #2 - MOISTURE of variety #1 = MOISTURE ADVANTAGE of variety #1.

10 NLFBLT = NORTHERN LEAF BLIGHT (*Helminthosporium turcicum* or *Exserohilum turcicum*). A 1 to 9 visual rating indicating the resistance to Northern Leaf Blight. A higher score indicates a higher resistance.

OILT = GRAIN OIL. Absolute value of oil content of the kernel as predicted by Near-Infrared Transmittance and expressed as a percent of dry matter.

15 PEDIGREE DISTANCE. Relationship among generations based on their ancestral links as evidenced in pedigrees. May be measured by the distance of the pedigree from a given starting point in the ancestry.

PERCENT IDENTITY. Percent identity as used herein refers to the comparison of the homozygous alleles of two inbred lines. Each inbred plant will have the same allele (and therefore be homozygous) at almost all of their loci. Percent identity is determined by comparing a statistically significant number of the 20 homozygous alleles of two inbred lines. For example, a percent identity of 90% between inbred PHCWK and other inbred line means that the two inbred lines have the same allele at 90% of their loci.

PERCENT SIMILARITY. Percent similarity as used herein refers to the comparison of the homozygous alleles of an inbred line with another plant. The 25 homozygous alleles of PHCWK are compared with the alleles of a non-inbred plant, such as a hybrid, and if the allele of the inbred matches at least one of the alleles from the hybrid then they are scored as similar. Percent similarity is determined by comparing a statistically significant number of loci and recording the number of loci with similar alleles as a percentage. For example, a percent similarity of 90% between inbred PHCWK and a hybrid maize plant means that the inbred line matches at least one of the hybrid alleles at 90% of the loci. In the case of a hybrid

produced from PHCWK as the male or female parent, such hybrid will comprise two sets of alleles, one set of which will comprise the same alleles as the homozygous alleles of inbred line PHCWK.

PLANT. As used herein, the term "plant" includes reference to an immature or 5 mature whole plant, including a plant that has been detasseled or from which seed or grain has been removed. Seed or embryo that will produce the plant is also considered to be the plant.

PLANT PARTS. As used herein, the term "plant parts" includes leaves, stems, roots, seed, grain, embryo, pollen, ovules, flowers, ears, cobs, husks, stalks, 10 root tips, anthers, pericarp, silk, tissue, cells and the like.

PLTHT = PLANT HEIGHT. This is a measure of the height of the plant from the ground to the tip of the tassel in centimeters.

POLSC = POLLEN SCORE. A 1 to 9 visual rating indicating the amount of pollen shed. The higher the score the more pollen shed.

15 POLWT = POLLEN WEIGHT. This is calculated by dry weight of tassels collected as shedding commences minus dry weight from similar tassels harvested after shedding is complete.

POP K/A = PLANT POPULATIONS. Measured as 1000s per acre.

20 POP ADV = PLANT POPULATION ADVANTAGE. The plant population advantage of variety #1 over variety #2 as calculated by PLANT POPULATION of variety #2 - PLANT POPULATION of variety #1 = PLANT POPULATION ADVANTAGE of variety #1.

PRM = PREDICTED RELATIVE MATURITY. This trait, predicted relative maturity, is based on the harvest moisture of the grain. The relative maturity rating is 25 based on a known set of checks and utilizes standard linear regression analyses and is also referred to as the Comparative Relative Maturity Rating System that is similar to the Minnesota Relative Maturity Rating System.

PRMSHD = A relative measure of the growing degree units (GDU) required to reach 50% pollen shed. Relative values are predicted values from the linear 30 regression of observed GDU's on relative maturity of commercial checks.

PROT = GRAIN PROTEIN. Absolute value of protein content of the kernel as predicted by Near-Infrared Transmittance and expressed as a percent of dry matter.

RTLDG = ROOT LODGING. Root lodging is the percentage of plants that do not root lodge; plants that lean from the vertical axis at an approximately 30 degree angle or greater would be counted as root lodged.

RTLADV = ROOT LODGING ADVANTAGE. The root lodging advantage of variety #1 over variety #2.

SCTGRN = SCATTER GRAIN. A 1 to 9 visual rating indicating the amount of scatter grain (lack of pollination or kernel abortion) on the ear. The higher the score the less scatter grain.

SDGVGR = SEEDLING VIGOR. This is the visual rating (1 to 9) of the amount of vegetative growth after emergence at the seedling stage (approximately five leaves). A higher score indicates better vigor.

SEL IND = SELECTION INDEX. The selection index gives a single measure of the hybrid's worth based on information for up to five traits. A maize breeder may utilize his or her own set of traits for the selection index. One of the traits that is almost always included is yield. The selection index data presented in the tables represent the mean value averaged across testing stations.

SELF POLLINATION. A plant is self-pollinated if pollen from one flower is transferred to the same or another flower of the same plant.

SIB POLLINATION. A plant is sib-pollinated when individuals within the same family or line are used for pollination.

SLFBLT = SOUTHERN LEAF BLIGHT (*Helminthosporium maydis* or *Bipolaris maydis*). A 1 to 9 visual rating indicating the resistance to Southern Leaf Blight. A higher score indicates a higher resistance.

SOURST = SOUTHERN RUST (*Puccinia polysora*). A 1 to 9 visual rating indicating the resistance to Southern Rust. A higher score indicates a higher resistance.

STAGRN = STAY GREEN. Stay green is the measure of plant health near the time of black layer formation (physiological maturity). A high score indicates better late-season plant health.

STDADV = STALK STANDING ADVANTAGE. The advantage of variety #1 over variety #2 for the trait STK CNT.

STKCNT = NUMBER OF PLANTS. This is the final stand or number of plants per plot.

5 STKLDG = STALK LODGING REGULAR. This is the percentage of plants that did not stalk lodge (stalk breakage) at regular harvest (when grain moisture is between about 20 and 30%) as measured by either natural lodging or pushing the stalks and determining the percentage of plants that break below the ear.

10 STKLDL = LATE STALK LODGING. This is the percentage of plants that did not stalk lodge (stalk breakage) at or around late season harvest (when grain moisture is between about 15 and 18%) as measured by either natural lodging or pushing the stalks and determining the percentage of plants that break below the ear.

15 STKLDS = STALK LODGING SCORE. A plant is considered as stalk lodged if the stalk is broken or crimped between the ear and the ground. This can be caused by any or a combination of the following: strong winds late in the season, disease pressure within the stalks, ECB damage or genetically weak stalks. This trait should be taken just prior to or at harvest. Expressed on a 1 to 9 scale with 9 being no lodging.

20 STLLPN = LATE STALK LODGING. This is the percent of plants that did not stalk lodge (stalk breakage or crimping) at or around late season harvest (when grain moisture is below 20%) as measured by either natural lodging or pushing the stalks and determining the percentage of plants that break or crimp below the ear.

25 STLPCN = STALK LODGING REGULAR. This is an estimate of the percentage of plants that did not stalk lodge (stalk breakage) at regular harvest (when grain moisture is between about 20 and 30%) as measured by either natural lodging or pushing the stalks and determining the percentage of plants that break below the ear.

STRT = GRAIN STARCH. Absolute value of starch content of the kernel as predicted by Near-Infrared Transmittance and expressed as a percent of dry matter.

30 STWWLT = Stewart's Wilt (*Erwinia stewartii*). A 1 to 9 visual rating indicating the resistance to Stewart's Wilt. A higher score indicates a higher resistance.

TASBLS = TASSEL BLAST. A 1 to 9 visual rating was used to measure the degree of blasting (necrosis due to heat stress) of the tassel at the time of flowering. A 1 would indicate a very high level of blasting at time of flowering, while a 9 would have no tassel blasting.

5 TASSZ = TASSEL SIZE. A 1 to 9 visual rating was used to indicate the relative size of the tassel. The higher the rating the larger the tassel.

TAS WT = TASSEL WEIGHT. This is the average weight of a tassel (grams) just prior to pollen shed.

10 TEXEAR = EAR TEXTURE. A 1 to 9 visual rating was used to indicate the relative hardness (smoothness of crown) of mature grain. A 1 would be very soft (extreme dent) while a 9 would be very hard (flinty or very smooth crown).

TILLER = TILLERS. A count of the number of tillers per plot that could possibly shed pollen was taken. Data are given as a percentage of tillers: number of tillers per plot divided by number of plants per plot.

15 TSTWT = TEST WEIGHT (UNADJUSTED). The measure of the weight of the grain in pounds for a given volume (bushel).

TSWADV = TEST WEIGHT ADVANTAGE. The test weight advantage of variety #1 over variety #2.

WIN M% = PERCENT MOISTURE WINS.

20 WIN Y% = PERCENT YIELD WINS.

YIELD BU/A = YIELD (BUSHELS/ACRE). Yield of the grain at harvest in bushels per acre adjusted to 15% moisture.

25 YLDADV = YIELD ADVANTAGE. The yield advantage of variety #1 over variety #2 as calculated by: YIELD of variety #1 - YIELD variety #2 = yield advantage of variety #1.

YLDSC = YIELD SCORE. A 1 to 9 visual rating was used to give a relative rating for yield based on plot ear piles. The higher the rating the greater visual yield appearance.

30 Definitions for Area of Adaptability

When referring to area of adaptability, such term is used to describe the location with the environmental conditions that would be well suited for this maize line. Area of adaptability is based on a number of factors, for example: days to maturity, insect resistance, disease resistance, and drought resistance. Area of adaptability does not indicate that the maize line will grow in every location within the area of adaptability or that it will not grow outside the area.

5 Central Corn Belt : Iowa, Illinois, Indiana

Drylands: non-irrigated areas of North Dakota, South Dakota, Nebraska, Kansas,

10 Colorado and Oklahoma

Eastern U.S.: Ohio, Pennsylvania, Delaware, Maryland, Virginia, and West Virginia

North central U.S.: Minnesota and Wisconsin

Northeast: Michigan, New York, Vermont, and Ontario and Quebec Canada

Northwest U.S.: North Dakota, South Dakota, Wyoming, Washington, Oregon,

15 Montana, Utah, and Idaho

South central U.S.: Missouri, Tennessee, Kentucky, Arkansas

Southeast U.S.: North Carolina, South Carolina, Georgia, Florida, Alabama,

Mississippi, and Louisiana

Southwest U.S.: Texas, Oklahoma, New Mexico, Arizona

20 Western U.S.: Nebraska, Kansas, Colorado, and California

Maritime Europe: Northern France, Germany, Belgium, Netherlands and Austria

## DETAILED DESCRIPTION OF THE INVENTION AND

## FURTHER EMBODIMENTS

25 All tables discussed in the Detailed Description of the Invention and Further Embodiments section can found at the end of the section.

### Morphological and Physiological Characteristics of PHCWK

Inbred maize line PHCWK is a yellow, dent maize inbred with some flint characteristics that may be used as either a male or female in the production of the first generation F1 maize hybrids although PHCWK may be best suited for use as a

female. Inbred maize line PHCWK is best adapted to the Northwest, Northcentral and Northeastern United States and Canada, and can be used to produce hybrids with approximately 91 maturity based on the Comparative Relative Maturity Rating System for harvest moisture of grain. Inbred maize line PHCWK demonstrates  
5 excellent general combining ability for yield, excellent roots, early flowering and high test weight as an inbred per se. In hybrid combination, inbred PHCWK demonstrates very high yielding, early flowering and fast drydown, excellent roots, test weight and brittle snap tolerance.

The inbred has shown uniformity and stability within the limits of environmental  
10 influence for all the traits as described in the Variety Description Information (Table 1, found at the end of the section). The inbred has been self-pollinated and ear-rowed a sufficient number of generations with careful attention paid to uniformity of plant type to ensure the homozygosity and phenotypic stability necessary for use in commercial hybrid seed production. The line has been increased both by hand and in isolated  
15 fields with continued observation for uniformity. No variant traits have been observed or are expected in PHCWK.

Inbred maize line PHCWK, being substantially homozygous, can be reproduced by planting seeds of the line, growing the resulting maize plants under self-pollinating or sib-pollinating conditions with adequate isolation, and harvesting  
20 the resulting seed using techniques familiar to the agricultural arts.

#### Genotypic Characteristics of PHCWK

In addition to phenotypic observations, a plant can also be identified by its  
25 genotype. The genotype of a plant can be characterized through a genetic marker profile, which can identify plants of the same variety or a related variety, or be used to determine or validate a pedigree. The SSR profile of Inbred PHCWK can be found in Table 2 at the end of this section.

As a result of inbreeding, PHCWK is substantially homozygous. This  
30 homozygosity has been characterized at the loci shown in the marker profile provided herein. An F1 hybrid made with PHCWK would comprise the marker profile of PHCWK shown herein. This is because an F1 hybrid is the sum of its inbred

parents, e.g., if one inbred parent is homozygous for allele x at a particular locus, and the other inbred parent is homozygous for allele y at that locus, the F1 hybrid will be x.y (heterozygous) at that locus. The profile can therefore be used to identify hybrids comprising PHCWK as a parent, since such hybrids will comprise two sets of alleles, 5 one set of which will be from PHCWK. The determination of the male set of alleles and the female set of alleles may be made by profiling the hybrid and the pericarp of the hybrid seed, which is composed of maternal parent cells. The paternal parent profile is obtained by subtracting the pericarp profile from the hybrid profile.

Subsequent generations of progeny produced by selection and breeding are 10 expected to be of genotype x (homozygous), y (homozygous), or x.y (heterozygous) for these locus positions. When the F1 plant is used to produce an inbred, the resulting inbred should be either x or y for that allele. In that regard, a unique allele or combination of alleles unique to that inbred can be used to identify progeny plants that retain those unique alleles or combinations of alleles.

Therefore, in accordance with the above, an embodiment of this invention is a PHCWK progeny maize plant or plant part that is a first generation hybrid maize plant comprising two sets of alleles, wherein one set of the alleles is the same as PHCWK at all of the SSR loci listed in Table 2. A maize cell wherein one set of the alleles is the same as PHCWK at all of the SSR loci listed in Table 2 is also an embodiment of the invention. This maize cell may be a part of a hybrid seed produced by crossing PHCWK with another inbred maize plant.

15 Genetic marker profiles can be obtained by techniques such as Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) which are also referred to as Microsatellites, and Single Nucleotide Polymorphisms (SNPs). For example, see Berry, Don, et al., "Assessing Probability 20 of Ancestry Using Simple Sequence Repeat Profiles: Applications to Maize Hybrids and Inbreds", Genetics, 2002, 161:813-824, and Berry, Don, et al., "Assessing Probability of Ancestry Using Simple Sequence Repeat Profiles: Applications to

Maize Inbred Lines and Soybean Varieties", Genetics, 2003, 165: 331-342, which are incorporated by reference herein.

Particular markers used for these purposes are not limited to the set of markers disclosed herein, but may include any type of marker and marker profile which provides a means of distinguishing varieties. In addition to being used for identification of Inbred Line PHCWK, a hybrid produced through the use of PHCWK, and the identification or verification of pedigree for progeny plants produced through the use of PHCWK, the genetic marker profile is also useful in further breeding and in developing an introgressed trait conversion of PHCWK.

Means of performing genetic marker profiles using SSR polymorphisms are well known in the art. SSRs are genetic markers based on polymorphisms in repeated nucleotide sequences, such as microsatellites. A marker system based on SSRs can be highly informative in linkage analysis relative to other marker systems in that multiple alleles may be present. Another advantage of this type of marker is that, through use of flanking primers, detection of SSRs can be achieved, for example, by the polymerase chain reaction (PCR), thereby eliminating the need for labor-intensive Southern hybridization. The PCR detection is done by use of two oligonucleotide primers flanking the polymorphic segment of repetitive DNA.

Repeated cycles of heat denaturation of the DNA followed by annealing of the primers to their complementary sequences at low temperatures, and extension of the annealed primers with DNA polymerase, comprise the major part of the methodology.

Following amplification, markers can be scored by electrophoresis of the amplification products. Scoring of marker genotype is based on the size of the amplified fragment, which may be measured by the base pair weight or molecular weight of the fragment. While variation in the primer used or in laboratory procedures can affect the reported fragment size, relative values should remain constant regardless of the specific primer or laboratory used. When comparing lines it is preferable if all SSR profiles are performed in the same lab. The SSR analyses reported herein were conducted in-house at Pioneer Hi-Bred. An SSR service is available to the public on a contractual basis by DNA Landmarks in Saint-Jean-sur-

Richelieu, Quebec, Canada (formerly Paragen, Celera AgGen, Perkin-Elmer AgGen, Linkage Genetics and NPI).

Primers used for the SSRs reported herein are publicly available and may be found in the Maize GDB on the World Wide Web at maizegdb.org (sponsored by the

5 USDA Agricultural Research Service), in Sharopova et al. (Plant Mol. Biol. 48(5-6):463-481), Lee et al (Plant Mol. Biol. 48(5-6); 453-461), or may be constructed from sequences if reported herein. Primers may be constructed from publicly available sequence information. Some marker information may also be available from DNA Landmarks.

10 Map information is provided by bin number as reported in the Maize GDB for the IBM 2 and/or IBM 2 Neighbors maps. The bin number digits to the left of decimal point represent the chromosome on which such marker is located, and the digits to the right of the decimal represent the location on such chromosome. A bin number.xx designation indicates that the bin location on that chromosome is not known. Map positions are also available on the Maize GDB for a variety of different

15 mapping populations.

PHCWK and its plant parts can be identified through molecular marker profile. An inbred corn plant cell having the SSR genetic marker profile shown in Table 2 is an embodiment of the invention. Such cell may be either diploid or haploid.

Also encompassed within the scope of the invention are plants and plant parts substantially benefiting from the use of PHCWK in their development, such as PHCWK comprising a introgressed trait through backcross conversion or transformation, and which may be identified by having an SSR molecular marker profile with a high percent identity to PHCWK, such as at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identity. Likewise, percent similarity at these percentages may be used to identify hybrid and other non-inbred plants produced by the use of PHCWK.

20

25

An embodiment of this invention is an inbred PHCWK progeny maize plant or plant part comprising the same homozygous alleles as the plant or plant part of PHCWK for at least 90% of the SSR loci listed in Table 2. A plant cell comprising

the same homozygous alleles as a plant cell of PHCWK for at least 90% of the SSR loci listed in Table 2 is also an embodiment of this invention. In these specific embodiments, 90% may also be replaced by any integer or partial integer percent of 80% or greater as listed above. One means of producing such a progeny plant, plant 5 part or cell is through the backcrossing and/or transformation methods described herein.

Similarly, an embodiment of this invention is a PHCWK progeny maize plant or plant part comprising at least one allele per locus that is the same allele as the plant or plant part of PHCWK for at least 90% of the SSR loci listed in Table 2. This 10 progeny plant may be a hybrid. A progeny or hybrid plant cell wherein at least one allele per locus that is the same allele as the plant cell PHCWK for at least 90% of the SSR loci listed in Table 2 is also a specific embodiment of this invention. In these specific embodiments, 90% may also be replaced by any integer percent listed above. One means of producing such a progeny or hybrid plant, plant part or cell is 15 through the backcrossing and/or transformation methods described herein.

In addition, the SSR profile of PHCWK also can be used to identify essentially derived varieties and other progeny lines developed from the use of PHCWK, as well as cells and other plant parts thereof. Progeny plants and plant parts produced using PHCWK may be identified by having a molecular marker profile of at least 25%, 30%, 20 35%, 40%, 45%, 50%, 55%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% genetic contribution from inbred line PHCWK, as measured by either percent identity or percent similarity.

25

## Comparing PHCWK To Other Inbreds

A breeder uses various methods to help determine which plants should be selected from segregating populations and ultimately which inbred lines will be used to develop hybrids for commercialization. In addition to knowledge of the germplasm 30 and plant genetics, a part of the selection process is dependent on experimental design coupled with the use of statistical analysis. Experimental design and statistical analysis are used to help determine which plants, which family of plants,

and finally which inbred lines and hybrid combinations are significantly better or different for one or more traits of interest. Experimental design methods are used to assess error so that differences between two inbred lines or two hybrid lines can be more accurately evaluated. Statistical analysis includes the calculation of mean

5 values, determination of the statistical significance of the sources of variation, and the calculation of the appropriate variance components. Either a five or a one percent significance level is customarily used to determine whether a difference that occurs for a given trait is real or due to the environment or experimental error. One of ordinary skill in the art of plant breeding would know how to evaluate the traits of

10 two plant varieties to determine if there is no significant difference between the two traits expressed by those varieties. For example, see Fehr, Walt, Principles of Cultivar Development, p. 261-286 (1987) which is incorporated herein by reference. Mean trait values may be used to determine whether trait differences are significant. Trait values should preferably be measured on plants grown under the same

15 environmental conditions, and environmental conditions should be appropriate for the traits or traits being evaluated. Sufficient selection pressure should be present for optimum measurement of traits of interest such as herbicide, insect or disease resistance. Similarly, an introgressed trait conversion of PHCWK for resistance, such as herbicide resistance, should not be compared to PHCWK in the presence of the

20 herbicide when comparing non-resistance related traits such as plant height and yield.

In Table 3, data from traits and characteristics of inbred maize line PHCWK per se are given and compared to other maize inbred lines and hybrids. The following are the results of these comparisons:

25 The results in Table 3A compare inbred PHCWK to inbred PH5TG. The results show inbred PHCWK has significantly higher yield, early growth rate and plant height compared to PH5TG.

The results in Table 3B compare inbred PHCWK to inbred PH1CN. The results show inbred PHCWK has a significantly larger plant height and a lower

30 number of growing degree units required to achieve 50% pollen shed when compared to inbred PH1CN.

The results in Table 3C compare inbred PHCWK to inbred PHAA0. The results show inbred PHCWK differs significantly from PHAA0 in a number of traits including yield, plant height and ear height.

5 The results in Table 3D compare inbred PHCWK to inbred PH75K. The results show inbred PHCWK differs significantly from PH75K in a number of traits including yield, plant height and ear height.

#### Development of maize hybrids using PHCWK

A single cross maize hybrid results from the cross of two inbred lines, each of  
10 which has a genotype that complements the genotype of the other. The hybrid progeny of the first generation is designated F1. In the development of commercial hybrids in a maize plant breeding program, only the F1 hybrid plants are sought. F1 hybrids are more vigorous than their inbred parents. This hybrid vigor, or heterosis, can be manifested in many polygenic traits, including increased vegetative growth  
15 and increased yield.

PHCWK may be used to produce hybrid maize. One such embodiment is the method of crossing inbred maize line PHCWK with another maize plant, such as a different maize inbred line, to form a first generation F1 hybrid seed. The first generation F1 hybrid seed, plant and plant part produced by this method is an  
20 embodiment of the invention. The first generation F1 seed, plant and plant part will comprise an essentially complete set of the alleles of inbred line PHCWK. One of ordinary skill in the art can utilize either breeder books or molecular methods to identify a particular F1 hybrid plant produced using inbred line PHCWK. Further, one of ordinary skill in the art may also produce F1 hybrids with transgenic, male sterile  
25 and/or backcross conversions of inbred line PHCWK.

The development of a maize hybrid in a maize plant breeding program involves three steps: (1) the selection of plants from various germplasm pools for initial breeding crosses; (2) the selfing of the selected plants from the breeding crosses for several generations to produce a series of inbred lines, such as PHCWK,  
30 which, although different from each other, breed true and are highly uniform; and (3) crossing the selected inbred lines with different inbred lines to produce the hybrids.

During the inbreeding process in maize, the vigor of the lines decreases, and so one would not be likely to use PHCWK directly to produce grain. However, vigor is restored when PHCWK is crossed to a different inbred line to produce a commercial F1 hybrid. An important consequence of the homozygosity and homogeneity of the 5 inbred line is that the hybrid between a defined pair of inbreds may be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained.

PHCWK may be used to produce a single cross hybrid, a three-way hybrid or a double cross hybrid. A single cross hybrid is produced when two inbred lines are crossed to produce the F1 progeny. A double cross hybrid is produced from four 10 inbred lines crossed in pairs (A x B and C x D) and then the two F1 hybrids are crossed again (A x B) x (C x D). A three-way cross hybrid is produced from three inbred lines where two of the inbred lines are crossed (A x B) and then the resulting F1 hybrid is crossed with the third inbred (A x B) x C. In each case, pericarp tissue from the female parent will be a part of and protect the hybrid seed.

15

### Combining Ability of PHCWK

Combining ability of a line, as well as the performance of the line per se, is a factor in the selection of improved maize inbreds. Combining ability refers to a line's 20 contribution as a parent when crossed with other lines to form hybrids. The hybrids formed for the purpose of selecting superior lines may be referred to as test crosses, and include comparisons to other hybrid varieties grown in the same environment (same cross, location and time of planting). One way of measuring combining ability is by using values based in part on the overall mean of a number of test crosses, 25 weighted by number of experiment and location combinations in which the hybrid combinations occurs. The mean may be adjusted to remove environmental effects and known genetic relationships among the lines.

General combining ability provides an overall score for the inbred over a large number of test crosses. Specific combining ability provides information on hybrid 30 combinations formed by PHCWK and a specific inbred parent. A line such as PHCWK which exhibits good general combining ability may be used in a large number of hybrid combinations.

A general combining ability report for inbred PHCWK is provided in Table 4. This data represents the overall mean value for these traits over hundreds of test crosses. Table 4 demonstrates that inbred PHCWK shows good general combining ability for hybrid production.

5

### Hybrid Comparisons

These hybrid comparisons represent specific hybrid crosses with PHCWK and a comparison of these specific hybrids with other hybrids with favorable characteristics. These comparisons illustrate the good specific combining ability of 10 PHCWK.

The results in Table 5A compare a specific hybrid for which inbred PHCWK is a parent and a second hybrid, 38R92. The results show that the hybrid containing inbred PHCWK produced significantly different results over multiple traits including moisture, plant height and ear height.

15 The results in Table 5B compare a specific hybrid for which inbred PHCWK is a parent and a second hybrid, 38G60. The results show that the hybrid containing inbred PHCWK produced significantly different results over multiple traits including test weight, moisture, and number of growing degree units required to achieve 50% pollen shed.

20 The results in Table 5C compare a specific hybrid for which inbred PHCWK is a parent and a second hybrid, 38R69. The results show that the hybrid containing inbred PHCWK produced significantly different results over multiple traits including plant height, late season health and test weight.

25 The results in Table 5D compare a specific hybrid for which inbred PHCWK is a parent and a second hybrid, 38R92. The results show that the hybrid containing inbred PHCWK produced significantly different results over multiple traits including plant height, moisture and ear height.

The results in Table 5E compare a specific hybrid for which inbred PHCWK is a parent and a second hybrid, 39K40. The results show that the hybrid containing 30 inbred PHCWK produced significantly different results over multiple traits including yield and moisture.

The results in Table 5F compare a specific hybrid for which inbred PHCWK is a parent and a second hybrid, 38G60. The results show that the hybrid containing inbred PHCWK produced numerically different results over multiple traits including ear height, plant height and number of growing degree units required to achieve 50% pollen shed.

The results in Table 5G compare a specific hybrid for which inbred PHCWK is a parent and a second hybrid, 38R69. The results show that the hybrid containing inbred PHCWK produced numerically different results over multiple traits including plant height, number of growing degree units required to achieve 50% pollen shed and number of plants per plot.

#### Introgression of a new locus or trait into PHCWK.

PHCWK represents a new base genetic line into which a new locus or trait may be introgressed. Direct transformation and backcrossing represent two important methods that can be used to accomplish such an introgression. The term backcross conversion and single locus conversion are used interchangeably to designate the product of a backcrossing program.

#### Backcross Conversions of PHCWK

A backcross conversion of PHCWK occurs when DNA sequences are introduced through backcrossing (Hallauer *et al*, 1988), with PHCWK utilized as the recurrent parent. Both naturally occurring and transgenic DNA sequences may be introduced through backcrossing techniques. A backcross conversion may produce a plant with a trait or locus conversion in at least one or more backcrosses, including at least 2 crosses, at least 3 crosses, at least 4 crosses, at least 5 crosses and the like. Molecular marker assisted breeding or selection may be utilized to reduce the number of backcrosses necessary to achieve the backcross conversion. For example, see Openshaw, S.J. *et al.*, Marker-assisted Selection in Backcross Breeding. In: Proceedings Symposium of the Analysis of Molecular Data, August 1994, Crop Science Society of America, Corvallis, OR, where it is demonstrated that a backcross conversion can be made in as few as two backcrosses.

The complexity of the backcross conversion method depends on the type of trait being transferred (single genes or closely linked genes as vs. unlinked genes), the level of expression of the trait, the type of inheritance (cytoplasmic or nuclear) and the types of parents included in the cross. It is understood by those of ordinary skill in the art that for single gene traits that are relatively easy to classify, the backcross method is effective and relatively easy to manage. (See Hallauer et al. in Corn and Corn Improvement, Sprague and Dudley, Third Ed. 1998). Desired traits that may be transferred through backcross conversion include, but are not limited to, waxy starch, sterility (nuclear and cytoplasmic), fertility restoration, grain color (white), nutritional enhancements, drought tolerance, nitrogen utilization, altered fatty acid profile, increased digestibility, low phytate, industrial enhancements, disease resistance (bacterial, fungal or viral), insect resistance, herbicide resistance and yield enhancements. In addition, an introgression site itself, such as an FRT site, Lox site or other site specific integration site, may be inserted by backcrossing and utilized for direct insertion of one or more genes of interest into a specific plant variety. In some embodiments of the invention, the number of loci that may be backcrossed into PHCWK is at least 1, 2, 3, 4, or 5 and/or no more than 6, 5, 4, 3, or 2. A single loci may contain several transgenes, such as a transgene for disease resistance that, in the same expression vector, also contains a transgene for herbicide resistance. The gene for herbicide resistance may be used as a selectable marker and/or as a phenotypic trait. A single locus conversion of site specific integration system allows for the integration of multiple genes at the converted loci.

The backcross conversion may result from either the transfer of a dominant allele or a recessive allele. Selection of progeny containing the trait of interest is accomplished by direct selection for a trait associated with a dominant allele. Transgenes transferred via backcrossing typically function as a dominant single gene trait and are relatively easy to classify. Selection of progeny for a trait that is transferred via a recessive allele, such as the waxy starch characteristic, requires growing and selfing the first backcross generation to determine which plants carry the recessive alleles. Recessive traits may require additional progeny testing in successive backcross generations to determine the presence of the locus of interest.

The last backcross generation is usually selfed to give pure breeding progeny for the gene(s) being transferred, although a backcross conversion with a stably introgressed trait may also be maintained by further backcrossing to the recurrent parent with selection for the converted trait.

5 Along with selection for the trait of interest, progeny are selected for the phenotype of the recurrent parent. While occasionally additional polynucleotide sequences or genes may be transferred along with the backcross conversion, the backcross conversion line “fits into the same hybrid combination as the recurrent parent inbred line and contributes the effect of the additional gene added through the  
10 backcross.” Poehlman et al. (1995, page 334). It has been proposed that in general there should be at least four backcrosses when it is important that the recovered lines be essentially identical to the recurrent parent except for the characteristic being transferred (Fehr 1987, Principles of Cultivar Development). However, as noted above, the number of backcrosses necessary can be reduced with the use of  
15 molecular markers. Other factors, such as a genetically similar donor parent, may also reduce the number of backcrosses necessary.

One process for adding or modifying a trait or locus in maize inbred line PHCWK comprises crossing PHCWK plants grown from PHCWK seed with plants of another maize line that comprise the desired trait or locus, selecting F1 progeny  
20 plants that comprise the desired trait or locus to produce selected F1 progeny plants, crossing the selected progeny plants with the PHCWK plants to produce backcross progeny plants, selecting for backcross progeny plants that have the desired trait or locus and the morphological characteristics of maize inbred line PHCWK to produce selected backcross progeny plants; and backcrossing to PHCWK three or more times  
25 in succession to produce selected fourth or higher backcross progeny plants that comprise said trait or locus. The modified PHCWK may be further characterized as having the physiological and morphological characteristics of maize inbred line PHCWK listed in Table 1 as determined at the 5% significance level when grown in the same environmental conditions and/or may be characterized by percent similarity  
30 or identity to PHCWK as determined by SSR markers. The above method may be utilized with fewer backcrosses in appropriate situations, such as when the donor

parent is highly related or markers are used in the selection step. Desired traits that may be used include those nucleic acids known in the art, some of which are listed herein, that will affect traits through nucleic acid expression or inhibition. Desired loci include the introgression of FRT, Lox and other sites for site specific integration,

5 which may also affect a desired trait if a functional nucleic acid is inserted at the integration site.

In addition, the above process and other similar processes described herein may be used to produce F1 hybrid maize seed by adding a step at the end of the process that comprises crossing PHCWK with the introgressed trait or locus with a

10 different maize plant and harvesting the resultant F1 hybrid maize seed.

#### Male Sterility and Hybrid Seed Production

Hybrid seed production requires elimination or inactivation of pollen produced by the female inbred parent. Incomplete removal or inactivation of the pollen

15 provides the potential for self-pollination. A reliable method of controlling male fertility in plants offers the opportunity for improved seed production.

PHCWK can be produced in a male-sterile form. There are several ways in which a maize plant can be manipulated so that it is male sterile. These include use of manual or mechanical emasculation (or detasseling), use of one or more genetic factors that confer male sterility, including cytoplasmic genetic and/or nuclear genetic male sterility, use of gametocides and the like. A male sterile inbred designated PHCWK may include one or more genetic factors, which result in cytoplasmic genetic and/or nuclear genetic male sterility. All of such embodiments are within the scope of the present claims. The male sterility may be either partial or complete male sterility.

25 Hybrid maize seed is often produced by a male sterility system incorporating manual or mechanical detasseling. Alternate strips of two maize inbreds are planted in a field, and the pollen-bearing tassels are removed from one of the inbreds (female). Provided that there is sufficient isolation from sources of foreign maize pollen, the ears of the detasseled inbred will be fertilized only from the other inbred

30 (male), and the resulting seed is therefore hybrid and will form hybrid plants.

The laborious detasseling process can be avoided by using cytoplasmic male-sterile (CMS) inbreds. Plants of a CMS inbred are male sterile as a result of genetic factors in the cytoplasm, as opposed to the nucleus, and so nuclear linked genes are not transferred during backcrossing. Thus, this characteristic is inherited exclusively

5 through the female parent in maize plants, since only the female provides cytoplasm to the fertilized seed. CMS plants are fertilized with pollen from another inbred that is not male-sterile. Pollen from the second inbred may or may not contribute genes that make the hybrid plants male-fertile, and either option may be preferred depending on the intended use of the hybrid. The same hybrid seed, a portion produced from

10 detasseled fertile maize and a portion produced using the CMS system, can be blended to insure that adequate pollen loads are available for fertilization when the hybrid plants are grown. CMS systems have been successfully used since the 1950's, and the male sterility trait is routinely backcrossed into inbred lines. See Wych, p. 585-586, 1998.

15 There are several methods of conferring genetic male sterility available, such as multiple mutant genes at separate locations within the genome that confer male sterility, as disclosed in U.S. Patents 4,654,465 and 4,727,219 to Brar et al. and chromosomal translocations as described by Patterson in U.S. Patents No. 3,861,709 and 3,710,511. In addition to these methods, Albertsen et al., U.S. Patent 20 No. 5,432,068, describe a system of nuclear male sterility which includes: identifying a gene which is critical to male fertility; silencing this native gene which is critical to male fertility; removing the native promoter from the essential male fertility gene and replacing it with an inducible promoter; inserting this genetically engineered gene back into the plant; and thus creating a plant that is male sterile because the inducible promoter is not "on" resulting in the male fertility gene not being transcribed. 25 Fertility is restored by inducing, or turning "on", the promoter, which in turn allows the gene that confers male fertility to be transcribed.

These, and the other methods of conferring genetic male sterility in the art, each possess their own benefits and drawbacks. Some other methods use a variety 30 of approaches such as delivering into the plant a gene encoding a cytotoxic substance associated with a male tissue specific promoter or an antisense system in

which a gene critical to fertility is identified and an antisense to that gene is inserted in the plant (see: Fabinjanski, et al. EPO 89/3010153.8 publication no. 329,308 and PCT application PCT/CA90/00037 published as WO 90/08828).

Another system for controlling male sterility makes use of gametocides.

5 Gametocides are not a genetic system, but rather a topical application of chemicals. These chemicals affect cells that are critical to male fertility. The application of these chemicals affects fertility in the plants only for the growing season in which the gametocide is applied (see Carlson, Glenn R., U.S. Patent Number: 4,936,904). Application of the gametocide, timing of the application and genotype specificity often  
10 limit the usefulness of the approach and it is not appropriate in all situations.

Incomplete control over male fertility may result in self-pollinated seed being unintentionally harvested and packaged with hybrid seed. This would typically be only female parent seed, because the male plant is grown in rows that are typically destroyed prior to seed development. Once the seed from the hybrid bag is planted,  
15 it is possible to identify and select these self-pollinated plants. These self-pollinated plants will be genetically equivalent to one of the inbred lines used to produce the hybrid. Though the possibility of inbred PHCWK being included in a hybrid seed bag exists, the occurrence is very low because much care is taken by seed companies to avoid such inclusions. It is worth noting that hybrid seed is sold to growers for the  
20 production of grain or forage and not for breeding or seed production. These self-pollinated plants can be identified and selected by one skilled in the art due to their less vigorous appearance for vegetative and/or reproductive characteristics, including shorter plant height, small ear size, ear and kernel shape, cob color, or other characteristics.

25 Identification of these self-pollinated lines can also be accomplished through molecular marker analyses. See, "The Identification of Female Selfs in Hybrid Maize: A Comparison Using Electrophoresis and Morphology", Smith, J.S.C. and Wych, R.D., Seed Science and Technology 14, pp. 1-8 (1995), the disclosure of which is expressly incorporated herein by reference. Through these technologies, the  
30 homozygosity of the self pollinated line can be verified by analyzing allelic composition at various loci along the genome. Those methods allow for rapid

identification of the invention disclosed herein. See also, "Identification of Atypical Plants in Hybrid Maize Seed by Postcontrol and Electrophoresis" Sarca, V. et al., Probleme de Genetica Teoritica si Aplicata Vol. 20 (1) p. 29-42.

An embodiment of this invention is a process for producing seed of PHCWK, comprising planting a collection of seed comprising seed of a hybrid, one of whose parents is inbred PHCWK, said collection also comprising seed of said inbred, growing plants from said collection of seed, identifying inbred parent plants, selecting said inbred parent plant; and controlling pollination to preserve the homozygosity of said inbred parent plant.

## 5 Transformation

The advent of new molecular biological techniques has allowed the isolation and characterization of genetic elements with specific functions, such as encoding specific protein products. Scientists in the field of plant biology developed a strong interest in engineering the genome of plants to contain and express foreign genetic elements, or additional, or modified versions of native or endogenous genetic elements in order to alter the traits of a plant in a specific manner. Any DNA sequences, whether from a different species or from the same species, that are inserted into the genome using transformation are referred to herein collectively as "transgenes". In some embodiments of the invention, a transformed variant of PHCWK may contain at least one transgene but could contain at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and/or no more than 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2. Over the last fifteen to twenty years several methods for producing transgenic plants have been developed, and the present invention also relates to transformed versions of the claimed inbred maize line PHCWK as well as hybrid combinations thereof.

Numerous methods for plant transformation have been developed, including biological and physical plant transformation protocols. See, for example, Miki *et al.*, "Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B.R. and Thompson, J.E. Eds. (CRC Press, Inc., Boca Raton, 1993) pages 67-88 and Armstrong, "The First Decade of Maize Transformation: A Review and Future Perspective" (Maydica 44:101-109, 1999) . In

addition, expression vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are available. See, for example, Gruber et al., "Vectors for Plant Transformation" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B.R. and Thompson, J.E. Eds. (CRC Press, Inc., Boca Raton, 5 1993) pages 89-119.

The most prevalent types of plant transformation involve the construction of an expression vector. Such a vector comprises a DNA sequence that contains a gene under the control of or operatively linked to a regulatory element, for example a promoter. The vector may contain one or more genes and one or more regulatory 10 elements.

A genetic trait which has been engineered into the genome of a particular maize plant using transformation techniques, could be moved into the genome of another line using traditional breeding techniques that are well known in the plant breeding arts. For example, a backcrossing approach is commonly used to move a 15 transgene from a transformed maize plant to an elite inbred line, and the resulting progeny would then comprise the transgene(s). Also, if an inbred line was used for the transformation then the transgenic plants could be crossed to a different inbred in order to produce a transgenic hybrid maize plant.

Various genetic elements can be introduced into the plant genome using 20 transformation. These elements include, but are not limited to genes; coding sequences; inducible, constitutive, and tissue specific promoters; enhancing sequences; and signal and targeting sequences. For example, see the traits, genes and transformation methods listed in U.S. Patent 6,118,055.

With transgenic plants according to the present invention, a foreign protein 25 can be produced in commercial quantities. Thus, techniques for the selection and propagation of transformed plants, which are well understood in the art, yield a plurality of transgenic plants that are harvested in a conventional manner, and a foreign protein then can be extracted from a tissue of interest or from total biomass. Protein extraction from plant biomass can be accomplished by known methods which 30 are discussed, for example, by Heney and Orr, *Anal. Biochem.* 114: 92-6 (1981).

A genetic map can be generated, primarily via conventional Restriction

Fragment Length Polymorphisms (RFLP), Polymerase Chain Reaction (PCR) analysis, Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphisms (SNP) that identifies the approximate chromosomal location of the integrated DNA molecule. For exemplary methodologies in this regard, see Glick and Thompson,  
5 METHODS IN PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY 269-284 (CRC Press, Boca Raton, 1993).

Wang et al. discuss "Large Scale Identification, Mapping and Genotyping of Single-Nucleotide Polymorphisms in the Human Genome", Science, 280:1077-1082, 1998, and similar capabilities are becoming increasingly available for the corn  
10 genome. Map information concerning chromosomal location is useful for proprietary protection of a subject transgenic plant. If unauthorized propagation is undertaken and crosses made with other germplasm, the map of the integration region can be compared to similar maps for suspect plants to determine if the latter have a common parentage with the subject plant. Map comparisons would involve  
15 hybridizations, RFLP, PCR, SSR and sequencing, all of which are conventional techniques. SNPs may also be used alone or in combination with other techniques.

Likewise, by means of the present invention, plants can be genetically engineered to express various phenotypes of agronomic interest. Through the transformation of maize the expression of genes can be modulated to enhance  
20 disease resistance, insect resistance, herbicide resistance, agronomic, grain quality and other traits. Transformation can also be used to insert DNA sequences which control or help control male-sterility. DNA sequences native to maize as well as non-native DNA sequences can be transformed into maize and used to modulate levels of native or non-native proteins. Various promoters, targeting sequences, enhancing  
25 sequences, and other DNA sequences can be inserted into the maize genome for the purpose of modulating the expression of proteins. Reduction of the activity of specific genes (also known as gene silencing, or gene suppression) is desirable for several aspects of genetic engineering in plants.

Many techniques for gene silencing are well known to one of skill in the art,  
30 including but not limited to antisense technology (see, e.g., Sheehy et al. (1988) PNAS USA 85:8805-8809; and U.S. Patent Nos. 5,107,065; 5,453, 566; and

5,759,829); co-suppression (e.g., Taylor (1997) *Plant Cell* 9:1245; Jorgensen (1990) *Trends Biotech.* 8(12):340-344; Flavell (1994) *PNAS USA* 91:3490-3496; Finnegan et al. (1994) *Bio/Technology* 12: 883-888; and Neuhuber et al. (1994) *Mol. Gen. Genet.* 244:230-241); RNA interference (Napoli et al. (1990) *Plant Cell* 2:279-289; 5 U.S. Patent No. 5,034,323; Sharp (1999) *Genes Dev.* 13:139-141; Zamore et al. (2000) *Cell* 101:25-33; and Montgomery et al. (1998) *PNAS USA* 95:15502-15507), virus-induced gene silencing (Burton, et al. (2000) *Plant Cell* 12:691-705; and Baulcombe (1999) *Curr. Op. Plant Bio.* 2:109-113); target-RNA-specific ribozymes (Haseloff et al. (1988) *Nature* 334: 585-591); hairpin structures (Smith et al. (2000) 10 *Nature* 407:319-320; WO 99/53050; and WO 98/53083); ribozymes (Steinecke et al. ((1992) *EMBO J.* 11:1525; and Perriman et al. ((1993) *Antisense Res. Dev.* 3:253); oligonucleotide mediated targeted modification (e.g., WO 03/076574 and WO 99/25853); Zn-finger targeted molecules (e.g., WO 01/52620; WO 03/048345; and WO 00/42219); and other methods or combinations of the above methods known to 15 those of skill in the art.

Exemplary transgenes useful for genetic engineering include, but are not limited to, those categorized below.

1. Transgenes That Confer Resistance To Pests or Disease And That Encode:

20 (A) Plant disease resistance genes. Plant defenses are often activated by specific interaction between the product of a disease resistance gene (R) in the plant and the product of a corresponding avirulence (Avr) gene in the pathogen. A plant variety can be transformed with cloned resistance gene to engineer plants that are resistant to specific pathogen strains. See, for example Jones et al., *Science* 266: 25 789 (1994) (cloning of the tomato Cf-9 gene for resistance to *Cladosporium fulvum*); Martin et al., *Science* 262: 1432 (1993) (tomato *Pto* gene for resistance to *Pseudomonas syringae* pv. tomato encodes a protein kinase); Mindrinos et al., *Cell* 78: 1089 (1994) (*Arabidopsis RSP2* gene for resistance to *Pseudomonas syringae*). A plant resistant to a disease is one that is more resistant to a pathogen as 30 compared to the wild type plant.

(B) A *Bacillus thuringiensis* protein, a derivative thereof or a synthetic

polypeptide modeled thereon. See, for example, Geiser *et al.*, *Gene* 48: 109 (1986), who disclose the cloning and nucleotide sequence of a *Bt* delta-endotoxin gene. Moreover, DNA molecules encoding delta-endotoxin genes can be purchased from American Type Culture Collection (Rockville, MD), for example, under ATCC 5 Accession Nos. 40098, 67136, 31995 and 31998. Other examples of *Bacillus thuringiensis* transgenes being genetically engineered are given in the following patents and patent applications and hereby are incorporated by reference for this purpose: 5,188,960; 5,689,052; 5,880,275; WO 91/114778; WO 99/31248; WO 01/12731; WO 99/24581; WO 97/40162 and US Application Serial Nos. 10/032,717; 10 10/414,637; and 10/606,320.

15 (C) An insect-specific hormone or pheromone such as an ecdysteroid and juvenile hormone, a variant thereof, a mimetic based thereon, or an antagonist or agonist thereof. See, for example, the disclosure by Hammock *et al.*, *Nature* 344: 458 (1990), of baculovirus expression of cloned juvenile hormone esterase, an inactivator of juvenile hormone.

20 (D) An insect-specific peptide which, upon expression, disrupts the physiology of the affected pest. For example, see the disclosures of Regan, *J. Biol. Chem.* 269: 9 (1994) (expression cloning yields DNA coding for insect diuretic hormone receptor), and Pratt *et al.*, *Biochem. Biophys. Res. Comm.* 163: 1243 (1989) (an allostatin is identified in *Diploptera punctata*). See also U.S. Patent No. 5,266,317 to Tomalski *et al.*, who disclose genes encoding insect-specific toxins.

(E) An enzyme responsible for an hyperaccumulation of a monterpene, a sesquiterpene, a steroid, hydroxamic acid, a phenylpropanoid derivative or another non-protein molecule with insecticidal activity.

25 (F) An enzyme involved in the modification, including the post-translational modification, of a biologically active molecule; for example, a glycolytic enzyme, a proteolytic enzyme, a lipolytic enzyme, a nuclease, a cyclase, a transaminase, an esterase, a hydrolase, a phosphatase, a kinase, a phosphorylase, a polymerase, an elastase, a chitinase and a glucanase, whether natural or synthetic. See PCT 30 application WO 93/02197 in the name of Scott *et al.*, which discloses the nucleotide sequence of a callase gene. DNA molecules which contain chitinase-encoding

sequences can be obtained, for example, from the ATCC under Accession Nos. 39637 and 67152. See also Kramer *et al.*, *Insect Biochem. Molec. Biol.* 23: 691 (1993), who teach the nucleotide sequence of a cDNA encoding tobacco hookworm chitinase, and Kawalleck *et al.*, *Plant Molec. Biol.* 21: 673 (1993), who provide the 5 nucleotide sequence of the parsley *ubi4-2* polyubiquitin gene.

(G) A molecule that stimulates signal transduction. For example, see the disclosure by Botella *et al.*, *Plant Molec. Biol.* 24: 757 (1994), of nucleotide sequences for mung bean calmodulin cDNA clones, and Griess *et al.*, *Plant Physiol.* 104: 1467 (1994), who provide the nucleotide sequence of a maize 10 calmodulin cDNA clone.

(H) A hydrophobic moment peptide. See PCT application WO 95/16776 (disclosure of peptide derivatives of Tachyplesin which inhibit fungal plant pathogens) and PCT application WO 95/18855 (teaches synthetic antimicrobial peptides that confer disease resistance), the respective contents of which are hereby incorporated 15 by reference for this purpose.

(I) A membrane permease, a channel former or a channel blocker. For example, see the disclosure by Jaynes *et al.*, *Plant Sci.* 89: 43 (1993), of heterologous expression of a cecropin-beta lytic peptide analog to render transgenic tobacco plants resistant to *Pseudomonas solanacearum*.

(J) A viral-invasive protein or a complex toxin derived therefrom. For example, the accumulation of viral coat proteins in transformed plant cells imparts resistance to viral infection and/or disease development effected by the virus from which the coat protein gene is derived, as well as by related viruses. See Beachy *et al.*, *Ann. Rev. Phytopathol.* 28: 451 (1990). Coat protein-mediated resistance has 25 been conferred upon transformed plants against alfalfa mosaic virus, cucumber mosaic virus, tobacco streak virus, potato virus X, potato virus Y, tobacco etch virus, tobacco rattle virus and tobacco mosaic virus. *Id.*

(K) An insect-specific antibody or an immunotoxin derived therefrom. Thus, an antibody targeted to a critical metabolic function in the insect gut would 30 inactivate an affected enzyme, killing the insect. Cf. Taylor *et al.*, Abstract #497, SEVENTH INT'L SYMPOSIUM ON MOLECULAR PLANT-MICROBE

INTERACTIONS (Edinburgh, Scotland, 1994) (enzymatic inactivation in transgenic tobacco via production of single-chain antibody fragments).

(L) A virus-specific antibody. See, for example, Tavladoraki *et al.*, *Nature* 366: 469 (1993), who show that transgenic plants expressing recombinant antibody genes are protected from virus attack.

(M) A developmental-arrestive protein produced in nature by a pathogen or a parasite. Thus, fungal endo alpha-1,4-D-polygalacturonases facilitate fungal colonization and plant nutrient release by solubilizing plant cell wall homo-alpha-1,4-D-galacturonase. See Lamb *et al.*, *Bio/Technology* 10: 1436 (1992). The cloning and characterization of a gene which encodes a bean endopolygalacturonase-inhibiting protein is described by Toubart *et al.*, *Plant J.* 2: 367 (1992).

(N) A developmental-arrestive protein produced in nature by a plant. For example, Logemann *et al.*, *Bio/Technology* 10: 305 (1992), have shown that transgenic plants expressing the barley ribosome-inactivating gene have an increased resistance to fungal disease.

(O) Genes involved in the Systemic Acquired Resistance (SAR) Response and/or the pathogenesis related genes. Briggs, S., *Current Biology*, 5(2) (1995).

(P) Antifungal genes (Cornelissen and Melchers, *Pl. Physiol.* 101:709-712, (1993) and Parijs *et al.*, *Planta* 183:258-264, (1991) and Bushnell *et al.*, *Can. J. of Plant Path.* 20(2):137-149 (1998).

(Q) Detoxification genes, such as for fumonisin, beauvericin, moniliformin and zearalenone and their structurally related derivatives. For example, see US Patent No. 5,792,931.

(R) Cystatin and cysteine proteinase inhibitors.

(S) Defensin genes. See WO03000863.

(T) Genes conferring resistance to nematodes. See WO 03/033651 and Urwin *et. al.*, *Planta* 204:472-479 (1998).

## 2. Transgenes That Confer Resistance To A Herbicide, For Example:

(A) A herbicide that inhibits the growing point or meristem, such as an imidazolinone or a sulfonylurea. Exemplary genes in this category code for mutant

ALS and AHAS enzyme as described, for example, by Lee *et al.*, *EMBO J.* 7: 1241 (1988), and Miki *et al.*, *Theor. Appl. Genet.* 80: 449 (1990), respectively. See also, U.S. Patent Nos. 5,605,011; 5,013,659; 5,141,870; 5,767,361; 5,731,180; 5,304,732; 4,761,373; 5,331,107; 5,928,937; and 5,378,824; and international publication WO 96/33270, which are incorporated herein by reference for this purpose.

(B) Glyphosate (resistance imparted by mutant 5-enolpyruvl-3-phosphikimate synthase (EPSP) and *aroA* genes, respectively) and other phosphono compounds such as glufosinate (phosphinothricin acetyl transferase (PAT) and *Streptomyces hygroscopicus* phosphinothricin acetyl transferase (*bar*) genes), and pyridinoxy or phenoxy propionic acids and cyclohexones (ACCase inhibitor-encoding genes). See, for example, U.S. Patent No. 4,940,835 to Shah *et al.*, which discloses the nucleotide sequence of a form of EPSPS which can confer glyphosate resistance. U.S. Patent No. 5,627,061 to Barry *et al.* also describes genes encoding EPSPS enzymes. See also U.S. Patent Nos. 6,248,876 B1; 6,040,497; 5,804,425; 5,633,435; 5,145,783; 4,971,908; 5,312,910; 5,188,642; 4,940,835; 5,866,775; 6,225,114 B1; 6,130,366; 5,310,667; 4,535,060; 4,769,061; 5,633,448; 5,510,471; Re. 36,449; RE 37,287 E; and 5,491,288; and international publications WO 97/04103; WO 97/04114; WO 00/66746; WO 01/66704; WO 00/66747 and WO 00/66748, which are incorporated herein by reference for this purpose. Glyphosate resistance is also imparted to plants that express a gene that encodes a glyphosate oxido-reductase enzyme as described more fully in U.S. Patent Nos. 5,776,760 and 5,463,175, which are incorporated herein by reference for this purpose. In addition glyphosate resistance can be imparted to plants by the over expression of genes encoding glyphosate N-acetyltransferase. See, for example, U.S. Application Serial Nos. 60/244,385; 60/377,175 and 60/377,719. A DNA molecule encoding a mutant *aroA* gene can be obtained under ATCC accession No. 39256, and the nucleotide sequence of the mutant gene is disclosed in U.S. Patent No. 4,769,061 to Comai. European patent application No. 0 333 033 to Kumada *et al.* and U.S. Patent No. 4,975,374 to Goodman *et al.* disclose nucleotide sequences of glutamine synthetase genes which confer resistance to herbicides such as L-phosphinothricin. The nucleotide sequence of a phosphinothricin-acetyl-transferase

gene is provided in European Patent No. 0 242 246 and 0 242 236 to Leemans *et al.* De Greef *et al.*, *Bio/Technology* 7: 61 (1989), describe the production of transgenic plants that express chimeric *bar* genes coding for phosphinothricin acetyl transferase activity. See also, U.S. Patent Nos. 5,969,213; 5,489,520; 5,550,318; 5,874,265; 5,919,675; 5,561,236; 5,648,477; 5,646,024; 6,177,616 B1; and 5,879,903, which are incorporated herein by reference for this purpose. Exemplary genes conferring resistance to phenoxy propionic acids and cyclohexones, such as sethoxydim and haloxyfop, are the *Acc1-S1*, *Acc1-S2* and *Acc1-S3* genes described by Marshall *et al.*, *Theor. Appl. Genet.* 83: 435 (1992).

(C) A herbicide that inhibits photosynthesis, such as a triazine (*psbA* and *gs+* genes) and a benzonitrile (nitrilase gene). Przibilla *et al.*, *Plant Cell* 3: 169 (1991), describe the transformation of *Chlamydomonas* with plasmids encoding mutant *psbA* genes. Nucleotide sequences for nitrilase genes are disclosed in U.S. Patent No. 4,810,648 to Stalker, and DNA molecules containing these genes are available under ATCC Accession Nos. 53435, 67441 and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes *et al.*, *Biochem. J.* 285: 173 (1992).

(D) Acetohydroxy acid synthase, which has been found to make plants that express this enzyme resistant to multiple types of herbicides, has been introduced into a variety of plants (see, e.g., Hattori *et al.* (1995) *Mol Gen Genet* 246:419). Other genes that confer tolerance to herbicides include: a gene encoding a chimeric protein of rat cytochrome P4507A1 and yeast NADPH-cytochrome P450 oxidoreductase (Shiota *et al.* (1994) *Plant Physiol Plant Physiol* 106:17), genes for glutathione reductase and superoxide dismutase (Aono *et al.* (1995) *Plant Cell Physiol* 36:1687, and genes for various phosphotransferases (Datta *et al.* (1992) *Plant Mol Biol* 20:619).

(E) Protoporphyrinogen oxidase (protox) is necessary for the production of chlorophyll, which is necessary for all plant survival. The protox enzyme serves as the target for a variety of herbicidal compounds. These herbicides also inhibit growth of all the different species of plants present, causing their total destruction. The development of plants containing altered protox activity which are resistant to these

herbicides are described in U.S. Patent Nos. 6,288,306 B1; 6,282,837 B1; and 5,767,373; and international publication WO 01/12825, which are incorporated herein by reference for this purpose.

5       3.      Transgenes That Confer Or Contribute To A Grain Trait, Such As:

(A)     Modified fatty acid metabolism, for example, by

(1)    Transforming a plant with an antisense gene of stearoyl-ACP desaturase to increase stearic acid content of the plant. See Knultzon *et al.*, *Proc. Natl. Acad. Sci. USA* 89: 2624 (1992),

10           (2)   Elevating oleic acid via FAD-2 gene modification and/or decreasing linolenic acid via FAD-3 gene modification (see U.S. Patents 6,063,947; 6,323,392; and WO 93/11245),

(3)    Altering conjugated linolenic or linoleic acid content, such as in WO 01/12800,

15           (4)   Modifying LEC1, AGP, Dek1, Superal1, thioredoxin, and/or a gamma zein knock out or mutant such as cs27 or TUSC 27. For example, see WO 02/42424, WO 98/22604, WO 03/011015, US 6,423,886 and Rivera-Madrid, R. et. al. *Proc. Natl. Acad. Sci.* 92:5620-5624 (1995).

20           (B)   Decreased phytate content, for example, by the

(1)    Introduction of a phytase-encoding gene would enhance breakdown of phytate, adding more free phosphate to the transformed plant. For example, see Van Hartingsveldt *et al.*, *Gene* 127: 87 (1993), for a disclosure of the nucleotide sequence of an *Aspergillus niger* phytase gene.

25           (2)   Introduction of a gene that reduces phytate content. In maize, this, for example, could be accomplished, by cloning and then re-introducing DNA associated with one or more of the alleles, such as the LPA alleles, identified in maize mutants characterized by low levels of phytic acid, such as in Raboy *et al.*, *Maydica* 35: 383 (1990) and/or by altering inositol kinase activity as in WO 02/059324, US2003/0009011, WO 03/027243, US2003/0079247 and WO 99/05298.

(C)    Modified carbohydrate composition effected, for example, by

transforming plants with a gene coding for an enzyme that alters the branching pattern of starch. See Shiroza *et al.*, *J. Bacteriol.* 170: 810 (1988) (nucleotide sequence of *Streptococcus mutans* fructosyltransferase gene), Steinmetz *et al.*, *Mol. Gen. Genet.* 200: 220 (1985) (nucleotide sequence of *Bacillus subtilis* levansucrase gene), Pen *et al.*, *Bio/Technology* 10: 292 (1992) (production of transgenic plants that express *Bacillus licheniformis* alpha-amylase), Elliot *et al.*, *Plant Molec. Biol.* 21: 515 (1993) (nucleotide sequences of tomato invertase genes), Søgaard *et al.*, *J. Biol. Chem.* 268: 22480 (1993) (site-directed mutagenesis of barley alpha-amylase gene), and Fisher *et al.*, *Plant Physiol.* 102: 1045 (1993) (maize endosperm starch branching enzyme II). The fatty acid modification genes mentioned above may also be used to effect starch content and/or composition through the interrelationship of the starch and oil pathways.

(D) Altered antioxidant content or composition, such as alteration of tocopherol or tocotrienols. For example, see WO 00/68393 involving the manipulation of antioxidant levels through alteration of a phytol prenyl transferase and WO 03/082899 through alteration of a homogentisate geranyl geranyl transferase.

(E) Improved digestibility and/or starch extraction through modification of UDP-D-xylose 4-epimerase, Fragile 1 and 2, Ref1, HCHL, C4H, such as in WO 99/10498.

20

4. Genes that Control Male-sterility

(A) Introduction of a deacetylase gene under the control of a tapetum-specific promoter and with the application of the chemical N-Ac-PPT (WO 01/29237).

25 (B) Introduction of various stamen-specific promoters (WO 92/13956, WO 92/13957).

(C) Introduction of the barnase and the barstar gene (Paul *et al.* *Plant Mol. Biol.* 19:611-622, 1992).

30 5. Genes that create a site for site specific DNA integration. This includes the introduction of FRT sites that may be used in the FLP/FRT system and/or Lox sites that may be used in the Cre/Loxp system. For example, see Lyznik, et al., Site-

Specific Recombination for Genetic Engineering in Plants, Plant Cell Rep (2003) 21:925-932 and WO 99/25821, which are hereby incorporated by reference. Other systems that may be used include the Gin recombinase of phage Mu (Maeser et al., 1991), the Pin recombinase of E. coli (Enomoto et al., 1983), and the R/RS system of the pSR1 plasmid (Araki et al., 1992).

6. Genes that affect growth characteristics, such as drought tolerance and nitrogen utilization. For example, see WO 00/73475 where water use efficiency is modulated through alteration of malate.

10

#### Using PHCWK to develop other maize inbreds

Inbred maize lines such as PHCWK are typically developed for use in the production of hybrid maize lines. However, inbred lines such as PHCWK also provide a source of breeding material that may be used to develop new maize inbred lines. Plant breeding techniques known in the art and used in a maize plant breeding program include, but are not limited to, recurrent selection, mass selection, bulk selection, mass selection, backcrossing, pedigree breeding, open pollination breeding, restriction fragment length polymorphism enhanced selection, genetic marker enhanced selection, making double haploids, and transformation. Often combinations of these techniques are used. The development of maize hybrids in a maize plant breeding program requires, in general, the development of homozygous inbred lines, the crossing of these lines, and the evaluation of the crosses. There are many analytical methods available to evaluate the result of a cross. The oldest and most traditional method of analysis is the observation of phenotypic traits but genotypic analysis may also be used.

#### Using PHCWK In A Breeding Program

This invention is directed to methods for producing a maize plant by crossing a first parent maize plant with a second parent maize plant wherein either the first or second parent maize plant is an inbred maize plant of the line PHCWK. The other parent may be any other maize plant, such as another inbred line or a plant that is

part of a synthetic or natural population. Any such methods using the inbred maize line PHCWK are part of this invention: selfing, sibbing, backcrosses, mass selection, pedigree breeding, bulk selection, hybrid production, crosses to populations, and the like. These methods are well known in the art and some of the more commonly used  
5 breeding methods are described below. Descriptions of breeding methods can also be found in one of several reference books (e.g., Allard, *Principles of Plant Breeding*, 1960; Simmonds, *Principles of Crop Improvement*, 1979; Fehr, "Breeding Methods for Cultivar Development", *Production and Uses*, 2<sup>nd</sup> ed., Wilcox editor, 1987).

10 Pedigree Breeding

Pedigree breeding starts with the crossing of two genotypes, such as PHCWK and one other elite inbred line having one or more desirable characteristics that is lacking or which complements PHCWK. If the two original parents do not provide all the desired characteristics, other sources can be included in the breeding population.  
15 In the pedigree method, superior plants are selfed and selected in successive filial generations. In the succeeding filial generations the heterozygous condition gives way to homogeneous lines as a result of self-pollination and selection. Typically in the pedigree method of breeding, five or more successive filial generations of selfing and selection is practiced: F1 → F2; F2 → F3; F3 → F4; F4 → F<sub>5</sub>, etc. After a  
20 sufficient amount of inbreeding, successive filial generations will serve to increase seed of the developed inbred. Preferably, the inbred line comprises homozygous alleles at about 95% or more of its loci.

In addition to being used to create a backcross conversion, backcrossing can also be used in combination with pedigree breeding to modify PHCWK and a hybrid  
25 that is made using the modified PHCWK. As discussed previously, backcrossing can be used to transfer one or more specifically desirable traits from one line, the donor parent, to an inbred called the recurrent parent, which has overall good agronomic characteristics yet lacks that desirable trait or traits. However, the same procedure can be used to move the progeny toward the genotype of the recurrent parent but at  
30 the same time retain many components of the non-recurrent parent by stopping the backcrossing at an early stage and proceeding with selfing and selection. For

example, an F1, such as a commercial hybrid, is created. This commercial hybrid may be backcrossed to one of its parent lines to create a BC1 or BC2. Progeny are selfed and selected so that the newly developed inbred has many of the attributes of the recurrent parent and yet several of the desired attributes of the non-recurrent 5 parent. This approach leverages the value and strengths of the recurrent parent for use in new hybrids and breeding.

Therefore, an embodiment of this invention is a method of making a backcross conversion of maize inbred line PHCWK, comprising the steps of crossing a plant of maize inbred line PHCWK with a donor plant comprising a mutant gene or transgene 10 conferring a desired trait, selecting an F1 progeny plant comprising the mutant gene or transgene conferring the desired trait, and backcrossing the selected F1 progeny plant to a plant of maize inbred line PHCWK. This method may further comprise the step of obtaining a molecular marker profile of maize inbred line PHCWK and using the molecular marker profile to select for a progeny plant with the desired trait and 15 the molecular marker profile of PHCWK. In the same manner, this method may be used to produce an F1 hybrid seed by adding a final step of crossing the desired trait conversion of maize inbred line PHCWK with a different maize plant to make F1 hybrid maize seed comprising a mutant gene or transgene conferring the desired trait.

20

#### Recurrent Selection and Mass Selection

Recurrent selection is a method used in a plant breeding program to improve a population of plants. PHCWK is suitable for use in a recurrent selection program. The method entails individual plants cross pollinating with each other to form 25 progeny. The progeny are grown and the superior progeny selected by any number of selection methods, which include individual plant, half-sib progeny, full-sib progeny, selfed progeny and topcrossing. The selected progeny are cross pollinated with each other to form progeny for another population. This population is planted and again superior plants are selected to cross pollinate with each other. Recurrent 30 selection is a cyclical process and therefore can be repeated as many times as desired. The objective of recurrent selection is to improve the traits of a population.

The improved population can then be used as a source of breeding material to obtain inbred lines to be used in hybrids or used as parents for a synthetic cultivar. A synthetic cultivar is the resultant progeny formed by the intercrossing of several selected inbreds.

5        Mass selection is a useful technique when used in conjunction with molecular marker enhanced selection. In mass selection seeds from individuals are selected based on phenotype and/or genotype. These selected seeds are then bulked and used to grow the next generation. Bulk selection requires growing a population of plants in a bulk plot, allowing the plants to self-pollinate, harvesting the seed in bulk  
10      and then using a sample of the seed harvested in bulk to plant the next generation. Instead of self pollination, directed pollination could be used as part of the breeding program.

### Mutation Breeding

15        Mutation breeding is one of many methods that could be used to introduce new traits into PHCWK. Mutations that occur spontaneously or are artificially induced can be useful sources of variability for a plant breeder. The goal of artificial mutagenesis is to increase the rate of mutation for a desired characteristic. Mutation rates can be increased by many different means including temperature, long-term  
20      seed storage, tissue culture conditions, radiation; such as X-rays, Gamma rays (e.g. cobalt 60 or cesium 137), neutrons, (product of nuclear fission by uranium 235 in an atomic reactor), Beta radiation (emitted from radioisotopes such as phosphorus 32 or carbon 14), or ultraviolet radiation (preferably from 2500 to 2900nm), or chemical mutagens (such as base analogues (5-bromo-uracil), related compounds (8-ethoxy  
25      caffeine), antibiotics (streptonigrin), alkylating agents (sulfur mustards, nitrogen mustards, epoxides, ethylenamines, sulfates, sulfonates, sulfones, lactones), azide, hydroxylamine, nitrous acid, or acridines. Once a desired trait is observed through mutagenesis the trait may then be incorporated into existing germplasm by traditional breeding techniques, such as backcrossing. Details of mutation breeding can be  
30      found in "Principals of Cultivar Development" Fehr, 1993 Macmillan Publishing Company the disclosure of which is incorporated herein by reference. In addition,

mutations created in other lines may be used to produce a backcross conversion of PHCWK that comprises such mutation.

#### Breeding with Molecular Markers

5 Molecular markers, which includes markers identified through the use of techniques such as Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs),  
10 Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs), may be used in plant breeding methods utilizing PHCWK.

Isozyme Electrophoresis and RFLPs as discussed in Lee, M., "Inbred Lines of Maize and Their Molecular Markers," *The Maize Handbook*, (Springer-Verlag, New York, Inc. 1994, at 423- 432), have been widely used to determine genetic composition. Isozyme Electrophoresis has a relatively low number of available markers and a low number of allelic variants among maize inbreds. RFLPs allow more discrimination because they have a higher degree of allelic variation in maize and a larger number of markers can be found. Both of these methods have been eclipsed by SSRs as discussed in Smith et al., "An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays L.*): comparisons with data from RFLPs and pedigree", *Theoretical and Applied Genetics* (1997) vol. 95 at 163-173 and by Pejic et al., "Comparative analysis of genetic similarity among maize inbreds detected by RFLPs, RAPDs, SSRs, and AFLPs," *Theoretical and Applied Genetics* (1998) at 25 1248-1255 incorporated herein by reference. SSR technology is more efficient and practical to use than RFLPs; more marker loci can be routinely used and more alleles per marker locus can be found using SSRs in comparison to RFLPs. Single Nucleotide Polymorphisms may also be used to identify the unique genetic composition of the invention and progeny lines retaining that unique genetic composition. Various molecular marker techniques may be used in combination to enhance overall resolution.

Maize DNA molecular marker linkage maps have been rapidly constructed and widely implemented in genetic studies. One such study is described in Boppenmaier, et al., "Comparisons among strains of inbreds for RFLPs", Maize Genetics Cooperative Newsletter, 65:1991, pg. 90, is incorporated herein by reference.

5 One use of molecular markers is Quantitative Trait Loci (QTL) mapping. QTL mapping is the use of markers, which are known to be closely linked to alleles that have measurable effects on a quantitative trait. Selection in the breeding process is based upon the accumulation of markers linked to the positive effecting alleles and/or the elimination of the markers linked to the negative effecting alleles from the plant's genome.

10 Molecular markers can also be used during the breeding process for the selection of qualitative traits. For example, markers closely linked to alleles or markers containing sequences within the actual alleles of interest can be used to select plants that contain the alleles of interest during a backcrossing breeding program. The markers can also be used to select for the genome of the recurrent parent and against the genome of the donor parent. Using this procedure can minimize the amount of genome from the donor parent that remains in the selected plants. It can also be used to reduce the number of crosses back to the recurrent parent needed in a backcrossing program. The use of molecular markers in the 15 selection process is often called genetic marker enhanced selection.

### Production of Double Haploids

20 The production of double haploids can also be used for the development of inbreds in the breeding program. For example, an F1 hybrid for which PHCWK is a parent can be used to produce double haploid plants. Double haploids are produced by the doubling of a set of chromosomes (1N) from a heterozygous plant to produce a completely homozygous individual. For example, see Wan et al., "Efficient Production of Doubled Haplod Plants Through Colchicine Treatment of Anther-Derived Maize Callus", Theoretical and Applied Genetics, 77:889-892, 1989 and 25 US2003/0005479. This can be advantageous because the process omits the 30

generations of selfing needed to obtain a homozygous plant from a heterozygous source.

Thus, an embodiment of this invention is a process for making a substantially homozygous PHCWK progeny plant by producing or obtaining a seed from the cross 5 of PHCWK and another maize plant and applying double haploid methods to the F1 seed or F1 plant or to any successive filial generation. Such methods decrease the number of generations required to produce an inbred with similar genetics or characteristics to PHCWK. See Bernardo, R. and Kahler, A.L., *Theor. Appl. Genet.* 102:986-992, 2001.

In particular, a process of making seed retaining the molecular marker profile of maize inbred line PHCWK is contemplated, such process comprising obtaining or producing F1 hybrid seed for which maize inbred line PHCWK is a parent, inducing doubled haploids to create progeny without the occurrence of meiotic segregation, obtaining the molecular marker profile of maize inbred line PHCWK, and selecting progeny that retain the molecular marker profile of PHCWK.

10

#### Use Of PHCWK In Tissue Culture

This invention is also directed to the use of PHCWK in tissue culture. As used herein, the term "tissue culture" includes plant protoplasts, plant cell tissue culture, cultured microspores, plant calli, plant clumps, and the like. As used herein, phrases 15 such as "growing the seed" or "grown from the seed" include embryo rescue, isolation of cells from seed for use in tissue culture, as well as traditional growing methods.

Duncan, Williams, Zehr, and Widholm, *Planta* (1985) 165:322-332 reflects that 97% of the plants cultured that produced callus were capable of plant regeneration. Subsequent experiments with both inbreds and hybrids produced 91% regenerable 20 callus that produced plants. In a further study in 1988, Songstad, Duncan & Widholm in *Plant Cell Reports* (1988), 7:262-265 reports several media additions that enhance regenerability of callus of two inbred lines. Other published reports also indicated that "nontraditional" tissues are capable of producing somatic embryogenesis and plant regeneration. K.P. Rao, et al., *Maize Genetics Cooperation Newsletter*, 60:64-25 65 (1986), refers to somatic embryogenesis from glume callus cultures and B.V.

Conger, et al., *Plant Cell Reports*, 6:345-347 (1987) indicates somatic embryogenesis from the tissue cultures of maize leaf segments. Thus, it is clear from the literature that the state of the art is such that these methods of obtaining plants are, and were, "conventional" in the sense that they are routinely used and  
5 have a very high rate of success.

Tissue culture of maize, including tassel/anther culture, is described in U.S. 2002/0062506A1 and European Patent Application, publication 160,390, each of which are incorporated herein by reference for this purpose. Maize tissue culture procedures are also described in Green and Rhodes, "Plant Regeneration in Tissue  
10 Culture of Maize," *Maize for Biological Research* (Plant Molecular Biology Association, Charlottesville, Virginia 1982, at 367-372) and in Duncan, et al., "The Production of Callus Capable of Plant Regeneration from Immature Embryos of Numerous *Zea Mays* Genotypes," 165 *Planta* 322-332 (1985). Thus, another aspect of this invention is to provide cells which upon growth and differentiation produce  
15 maize plants having the genotype and/or physiological and morphological characteristics of inbred line PHCWK.

#### Progeny plants

All plants produced by the use of the methods described herein and that retain  
20 the unique genetic or trait combinations of PHCWK are within the scope of the invention. Progeny of the breeding methods described herein may be characterized in any number of ways, such as by traits retained in the progeny, pedigree and/or molecular markers. Combinations of these methods of characterization may be used.

Breeder's of ordinary skill in the art have developed the concept of an "essentially derived variety", which is defined in 7 U.S.C. § 2104(a)(3) of the Plant Variety Protection Act and is hereby incorporated by reference. Varieties and plants that are essentially derived from PHCWK are within the scope of the invention.

Pedigree is a method used by breeders of ordinary skill in the art to describe  
30 the varieties. Varieties that are more closely related by pedigree are likely to share common genotypes and combinations of phenotypic characteristics. All breeders of

ordinary skill in the art maintain pedigree records of their breeding programs. These pedigree records contain a detailed description of the breeding process, including a listing of all parental lines used in the breeding process and information on how such line was used. One embodiment of this invention is progeny plants and parts thereof  
5 with at least one ancestor that is PHCWK, and more specifically, where the pedigree of the progeny includes 1, 2, 3, 4, and/or 5 or less breeding crosses to a maize plant other than PHCWK or a plant that has PHCWK as a parent or other progenitor. A breeder of ordinary skill in the art would know if PHCWK were used in the development of a progeny line, and would also know how many crosses to a line  
10 other than PHCWK or line with PHCWK as a parent or other progenitor were made in the development of any progeny line.

Molecular markers also provide a means by which those of ordinary skill in the art characterize the similarity or differences of two lines. Using the breeding methods described herein, one can develop individual plants, plant cells, and populations of  
15 plants that retain at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% genetic contribution from inbred line PHCWK, as measured by either percent identity or percent similarity.  
20 In pedigree analysis the percentage genetic contribution may not be actually known, but on average 50% of the starting germplasm would be expected to be passed to the progeny line after one cross to another line, 25% after another cross to a different line, and so on. With backcrossing, the expected contribution of PHCWK after 2, 3, 4 and 5 doses (or 1, 2, 3 and 4 backcrosses) would be 75%, 87.5%, 93.75% and  
25 96.875% respectively. Actual genetic contribution may be much higher than the genetic contribution expected by pedigree, especially if molecular markers are used in selection. Molecular markers could also be used to confirm and/or determine the pedigree of the progeny line.

Traits are also used by those of ordinary skill in the art to characterize  
30 progeny. Traits are commonly evaluated at a significance level, such as a 1%, 5% or 10% significance level, when measured in plants grown in the same environmental

conditions. For example, a backcross conversion of PHCWK may be characterized as having the same morphological and physiological traits as PHCWK. The traits used for comparison may be any or all of the traits shown in Table 1, Table 3, Table 4 or Table 5.

5 A breeder will commonly work to combine a specific trait of an undeveloped variety of the species, such as a high level of resistance to a particular disease, with one or more of the elite agronomic characteristics (yield, maturity, plant size, lodging resistance, etc.) needed for use as a commercial variety. This combination, once developed, provides a valuable source of new germplasm for further breeding. For  
10 example, it may take 10-15 years and significant effort to produce such a combination, yet progeny may be developed that retain this combination in as little as 2-5 years and with much less effort.

#### Specific Embodiments

15 Specific methods and products produced using inbred line PHCWK in plant breeding are discussed in the following sections. The methods outlined are described in detail by way of illustration and example for purposes of clarity and understanding. However, it will be obvious that certain changes and modifications may be practiced within the scope of the invention.

20 One method for producing a line derived from inbred line PHCWK is as follows. One of ordinary skill in the art would produce or obtain a seed from the cross between inbred line PHCWK and another variety of maize, such as an elite inbred variety. The F1 seed derived from this cross would be grown to form a homogeneous population. The F1 seed would contain essentially all of the alleles  
25 from variety PHCWK and essentially all of the alleles from the other maize variety. The F1 nuclear genome would be made-up of 50% variety PHCWK and 50% of the other elite variety. The F1 seed would be grown and allowed to self, thereby forming F2 seed. On average the F2 seed would have derived 50% of its alleles from variety PHCWK and 50% from the other maize variety, but many individual plants from the population would have a greater percentage of their alleles derived from PHCWK  
30 (Wang J. and R. Bernardo, 2000, Crop Sci. 40:659-665 and Bernardo, R. and A.L.

Kahler, 2001, Theor. Appl. Genet 102:986-992). The molecular markers of PHCWK could be used to select and retain those lines with high similarity to PHCWK. The F2 seed would be grown and selection of plants would be made based on visual observation, markers and/or measurement of traits. The traits used for selection may

5 be any PHCWK trait described in this specification, including the inbred per se maize PHCWK traits described herein under the detailed description of inbred PHCWK. Such traits may also be the good general or specific combining ability of PHCWK, including its ability to produce hybrids with the approximate maturity and/or hybrid combination traits described herein under the detailed description of inbred PHCWK.

10 The PHCWK progeny plants that exhibit one or more of the desired PHCWK traits, such as those listed herein, would be selected and each plant would be harvested separately. This F3 seed from each plant would be grown in individual rows and allowed to self. Then selected rows or plants from the rows would be harvested individually. The selections would again be based on visual observation, markers

15 and/or measurements for desirable traits of the plants, such as one or more of the desirable PHCWK traits listed herein. The process of growing and selection would be repeated any number of times until a PHCWK progeny inbred plant is obtained. The PHCWK progeny inbred plant would contain desirable traits derived from inbred plant PHCWK, some of which may not have been expressed by the other maize

20 variety to which inbred line PHCWK was crossed and some of which may have been expressed by both maize varieties but now would be at a level equal to or greater than the level expressed in inbred variety PHCWK. However, in each case the resulting progeny line would benefit from the efforts of the inventor(s), and would not have existed but for the inventor(s) work in creating PHCWK. The PHCWK progeny

25 inbred plants would have, on average, 50% of their nuclear genes derived from inbred line PHCWK, but many individual plants from the population would have a greater percentage of their alleles derived from PHCWK. This breeding cycle, of crossing and selfing, and optional selection, may be repeated to produce another population of PHCWK progeny maize plants with, on average, 25% of their nuclear

30 genes derived from inbred line PHCWK, but, again, many individual plants from the population would have a greater percentage of their alleles derived from PHCWK.

This process can be repeated for a third, fourth, fifth, sixth, seventh or more breeding cycles. Another embodiment of the invention is a PHCWK progeny plant that has received the desirable PHCWK traits listed herein through the use of PHCWK, which traits were not exhibited by other plants used in the breeding process.

Therefore, an embodiment of this invention is a PHCWK progeny maize plant, wherein at least one ancestor of said PHCWK progeny maize plant is the maize plant or plant part of PHCWK, and wherein the pedigree of said PHCWK progeny maize plant is within two breeding crosses of PHCWK or a plant that has PHCWK as a parent. The progeny plants, parts and plant cells produced from PHCWK may be further characterized as having a percent marker similarity or identity with PHCWK as described herein.

5       The previous example can be modified in numerous ways, for instance selection may or may not occur at every selfing generation, selection may occur before or after the actual self-pollination process occurs, or individual selections may be made by harvesting individual ears, plants, rows or plots at any point during the breeding process described. Double haploid breeding methods may be used at any  
10 step in the process. Instead of selfing out of the hybrid produced from the inbred, one could first cross the hybrid to either a parent line or a different inbred, and then self out of that cross.

The population of plants produced at each and any cycle of breeding is also an embodiment of the invention, and on average each such population would  
15 predictably consist of plants containing approximately 50% of its genes from inbred line PHCWK in the first breeding cycle, 25% of its genes from inbred line PHCWK in the second breeding cycle, 12.5% of its genes from inbred line PHCWK in the third breeding cycle, 6.25% in the fourth breeding cycle, 3.125% in the fifth breeding cycle, and so on. However, in each case the use of PHCWK provides a substantial benefit.  
20 The linkage groups of PHCWK would be retained in the progeny lines, and since current estimates of the maize genome size is about 50,000-80,000 genes (Xiaowu, Gai et al., Nucleic Acids Research, 2000, Vol. 28, No. 1, 94-96), in addition to non-coding DNA that impacts gene expression, it provides a significant advantage to use

PHCWK as starting material to produce a line that retains desired genetics or traits of PHCWK.

Therefore, an embodiment of the invention is a process for making a population of PHCWK progeny inbred maize plants comprising obtaining or producing a first generation progeny maize seed comprising the plant of PHCWK as a parent, growing said first generation progeny maize seed to produce first generation maize plants and obtaining self or sib pollinated seed from said first generation maize plants, and growing the self or sib pollinated seed to obtain a population of PHCWK progeny inbred maize plants.

The population of PHCWK progeny inbred maize plants produced by this method are also embodiments of the invention, and such population as a whole will retain the expected genetic contribution of PHCWK. An inbred line selected from the population of PHCWK progeny inbred maize plants produced by this method is an embodiment, and such line may be further characterized by its molecular marker identity or similarity to PHCWK.

In this manner, the invention also encompasses a process for making a PHCWK inbred progeny maize plant comprising the steps of obtaining or producing a first generation progeny maize seed wherein a parent of said first generation progeny maize seed is a PHCWK plant, growing said first generation progeny maize seed to produce a first generation maize plant and obtaining self or sib pollinated seed from said first generation maize plant, and producing successive filial generations to obtain a PHCWK inbred progeny maize plant. Also an embodiment of this invention is the first breeding cycle inbred PHCWK maize plant produced by this method.

#### Crosses to other species

The utility of inbred maize line PHCWK also extends to crosses with other species. Commonly, suitable species will be of the family Graminaceae, and especially of the genera *Zea*, *Tripsacum*, *Coix*, *Schlerachne*, *Polytoca*, *Chionachne*, and *Trilobachne*, of the tribe Maydeae. Potentially suitable for crosses with PHCWK may be the various varieties of grain sorghum, *Sorghum bicolor* (L.) Moench.

## Industrial Applicability

Maize is used as human food, livestock feed, and as raw material in industry. The food uses of maize, in addition to human consumption of maize kernels, include both products of dry- and wet-milling industries. The principal products of maize dry milling are grits, meal and flour. The maize wet-milling industry can provide maize starch, maize syrups, and dextrose for food use. Maize oil is recovered from maize germ, which is a by-product of both dry- and wet-milling industries.

Maize, including both grain and non-grain portions of the plant, is also used extensively as livestock feed, primarily for beef cattle, dairy cattle, hogs, and poultry.

Industrial uses of maize include production of ethanol, maize starch in the wet-milling industry and maize flour in the dry-milling industry. The industrial applications of maize starch and flour are based on functional properties, such as viscosity, film formation, adhesive properties, and ability to suspend particles. The maize starch and flour have application in the paper and textile industries. Other industrial uses include applications in adhesives, building materials, foundry binders, laundry starches, explosives, oil-well muds, and other mining applications.

Plant parts other than the grain of maize are also used in industry: for example, stalks and husks are made into paper and wallboard and cobs are used for fuel and to make charcoal.

The seed of inbred maize line PHCWK, the plant produced from the inbred seed, the hybrid maize plant produced from the crossing of the inbred, hybrid seed, and various parts of the hybrid maize plant and transgenic versions of the foregoing, can be utilized for human food, livestock feed, and as a raw material in industry.

## DEPOSITS

Applicant will make a deposit of at least 2500 seeds of Inbred Maize Line PHCWK with the American Type Culture Collection (ATCC), Manassas, VA 20110 USA, ATCC Deposit No. \_\_\_\_\_. The seeds to be deposited with the ATCC on \_\_\_\_\_

5 will be taken from the deposit maintained by Pioneer Hi-Bred International, Inc., 800 Capital Square, 400 Locust Street, Des Moines, Iowa 50309-2340 since prior to the filing date of this application. Access to this deposit will be available during the pendency of the application to the Commissioner of Patents and Trademarks and persons determined by the Commissioner to be entitled thereto upon request. Upon

10 allowance of any claims in the application, the Applicant will make the deposit available to the public pursuant to 37 C.F.R. 1.808. This deposit of the Inbred Maize Line PHCWK will be maintained in the ATCC depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the enforceable life of the patent, whichever is longer, and will be replaced if it becomes

15 nonviable during that period. Additionally, Applicant has or will satisfy all of the requirements of 37 C.F.R. §§1.801 - 1.809, including providing an indication of the viability of the sample upon deposit. Applicant has no authority to waive any restrictions imposed by law on the transfer of biological material or its transportation in commerce. Applicant does not waive any infringement of rights granted under this

20 patent or under the Plant Variety Protection Act (7 USC 2321 et seq.). U.S. Plant Variety Protection of Inbred Maize Line PHCWK has been applied for.

## TABLES

TABLE 1  
VARIETY DESCRIPTION INFORMATION  
VARIETY = PHCWK

			Avg	StDev	N
1.	TYPE: (Describe intermediate types in comments section) 1=Sweet,2=Dent,3=Flint,4=Flour,5=Pop and 6=Ornamental. Comments: Flint Like			2	
5	2. MATURITY: DAYS HEAT UNITS Emergence to 50% of plants in silk Emergence to 50% of plants in pollen shed 10% to 90% pollen shed 50% Silk to harvest at 25% moisture	Days	H.Units		
		52	1,106		
		52	1,098		
		1	25		
10	3. PLANT: Plant Height (to tassel tip) (cm) Ear Height (to base of top ear node) (cm) Length of Top Ear Internode (cm) Average Number of Tillers per Plant	223.6	29.25	15	
		94.5	10.89	15	
		14.4	1.72	15	
15	Average Number of Ears per Stalk Anthocyanin of Brace Roots: 1=Absent, 2=Faint, 3=Moderate, 4=Dark	1.1	0.10	3	
			3		
20	4. LEAF: Width of Ear Node Leaf (cm) Length of Ear Node Leaf (cm) Number of Leaves above Top Ear Leaf Angle: (at anthesis, 2nd leaf above ear to stalk above leaf) (Degrees)	8.3	0.96	15	
		66.5	5.33	15	
		5.0	0.38	15	
		26.0	4.57	15	
25	* Leaf Color: Dark Green Munsell: 7.5GY44 Leaf Sheath Pubescence: 1=none to 9=like peach fuzz			3	
30	5. TASSEL: Number of Primary Lateral Branches Branch Angle from Central Spike Tassel Length: (from peduncle node to tassel tip),(cm). Pollen Shed: 0=male sterile, 9=heavy shed	4.5	1.25	15	
		26.3	3.71	15	
		48.5	3.70	15	
		4			
	* Anther Color: Light Green Munsell: 2.5GY78				
	* Glume Color: Med. Green Munsell: 5GY78				
	* Bar Glumes (glume bands): 1=absent, 2=present		1		
35	Peduncle Length: (from top leaf node to lower florets or branches),(cm).	18.5	3.64	15	
	* Munsell Glossy Book of Color, (A standard color reference). Kollmorgen Inst. Corp. New Windsor, NY.				

TABLE 1 VARIETY DESCRIPTION INFORMATION  
PHCWK

	6a. EAR (Unhusked ear)		AVG	STDEV	N
5	* Silk color: Pale Yellow (3 days after silk emergence)	Munsell: 7.5Y8.56			
	* Fresh husk color: Med. Green	Munsell: 7.5GY56			
10	* Dry husk color: Buff (65 days after 50% silking)	Munsell: 2.5Y8.54			
	Ear position at dry husk stage: 1=upright ,2=horizontal, 3=pendant		2		
	Husk Tightness: (1=very loose, 9=very tight)		5		
	Husk Extension (at harvest):1=short(ears exposed), 2=medium (<8cm), 3=long (8-10cm), 4=v. long (>10cm)		2		
15	6b. EAR (Husked ear data)				
	Ear Length (cm):	.	16.7	0.49	15
	Ear Diameter at mid-point (mm)		37.4	1.18	15
	Ear Weight (gm):		119.9	8.19	15
	Number of Kernel Rows:		12.7	1.45	15
20	Kernel Rows: 1=indistinct, 2=distinct		2		
	Row Alignment: 1=straight, 2=slightly curved, 3=spiral		2		
	Shank Length (cm):		13.1	1.33	15
	Ear Taper: 1=slight cylind., 2=average, 3=extreme		2		
25	7. KERNEL (Dried):				
	Kernel Length (mm):		10.1	0.46	15
	Kernel Width (mm):		8.1	0.35	15
	Kernel Thickness (mm):		4.8	0.68	15
	Round Kernels (shape grade) (%)		43.7	8.72	3
	Aleurone Color Pattern: 1=homozygous, 2=segregating		1		
30	* Aleurone Color: Yellow Munsell: 10YR612				
	* Hard Endo. Color: Yellow Munsell: 10YR610				
	Endosperm Type:		3		
	1=sweet (su1), 2=extra sweet (sh2), 3=normal starch, 4=high amylose starch, 5=waxy starch, 6=high protein, 7=high lysine, 8=super sweet (se), 9=high oil, 10=other				
35	Weight per 100 Kernels (unsized sample) (gm):		25.7	0.58	3
40	8. COB:				
	* Cob Diameter at mid-point (mm):		21.9	1.06	15
	* Cob Color: Red Munsell: 2.5YR38				
	* Munsell Glossy Book of color, (A standard color reference). Kollmorgen Inst. Corp. New Windsor, NY.				

TABLE 1 VARIETY DESCRIPTION INFORMATION  
PHCWK

**10. DISEASE RESISTANCE:**

(Rate from 1=most-susceptable to 9=most-resistant. Leave blank if not tested, leave race or strain options blank if polygenic.)

**A. LEAF BLIGHTS, WILTS, AND LOCAL INFECTION DISEASES**

Anthracnose Leaf Blight (*Colletotrichum graminicola*)

Common Rust (*Puccinia sorghi*)

Common Smut (*Ustilago maydis*)

10 7 Eyespot (*Kabatiella zeae*)

Gross's Wilt (*Clavibacter michiganense* spp. *nebraskense*)

4 Gray Leaf Spot (*Cercospora zeae-maydis*)

Helminthosporium Leaf Spot (*Bipolaris zeicola*) Race:

6 Northern Leaf Blight (*Exserohilum turcicum*) Race:

Southern Leaf Blight (*Bipolaris maydis*) Race:

Southern Rust (*Puccinia polyspora*)

Stewart's Wilt (*Erwinia stewartii*)

Other (Specify): \_\_\_\_\_

**B. SYSTEMIC DISEASES**

20 Corn Lethal Necrosis (MCMV and MDMV)

9 Head Smut (*Sphacelotheca reiliana*)

Maize Chlorotic Dwarf Virus (MDV)

Maize Chlorotic Mottle Virus (MCMV)

7 Maize Dwarf Mosaic Virus (MDMV)

25 Sorghum Downy Mildew of Corn (*Peronosclerospora sorghi*)

Other (Specify): \_\_\_\_\_

**C. STALK ROTS**

4 Anthracnose Stalk Rot (*Colletotrichum graminicola*)

Diplodia Stalk Rot (*Stenocarpella maydis*)

30 Fusarium Stalk Rot (*Fusarium moniliforme*)

Gibberella Stalk Rot (*Gibberella zeae*)

Other (Specify): \_\_\_\_\_

**D. EAR AND KERNEL ROTS**

Aspergillus Ear and Kernel Rot (*Aspergillus flavus*)

35 4 Diplodia Ear Rot (*Stenocarpella maydis*)

Fusarium Ear and Kernel Rot (*Fusarium moniliforme*)

Gibberella Ear Rot (*Gibberella zeae*)

Other (Specify): \_\_\_\_\_

TABLE 1

VARIETY DESCRIPTION INFORMATION  
PHCWK

**11. INSECT RESISTANCE:**

(Rate from 1=most-suscept. to 9=most-resist., leave blank if not tested.)

- 5      Corn Worm (*Helicoverpa zea*)  
       \_\_\_\_ Leaf Feeding  
       \_\_\_\_ Silk Feeding  
       \_\_\_\_ Ear Damage
- 10     Corn Leaf Aphid (*Rophalosiphum maydis*)  
     Corn Sap Beetle (*Capophilus dimidiatus*)  
     European Corn Borer (*Ostrinia nubilalis*)  
     1st. Generation (Typically whorl leaf feeding)  
     2nd. Generation (Typically leaf sheath-collar feeding)  
       \_\_\_\_ Stalk Tunneling  
       \_\_\_\_ cm tunneled/plant
- 15     Fall armyworm (*Spodoptera frugiperda*)  
       \_\_\_\_ Leaf Feeding  
       \_\_\_\_ Silk Feeding  
       \_\_\_\_ mg larval wt.
- 20     Maize Weevil (*Sitophilus zeamaize*)  
     Northern Rootworm (*Diabrotica barberi*)  
     Southern Rootworm (*Diabrotica undecimpunctata*)  
     Southwestern Corn Borer (*Diatraea grandiosella*)  
       \_\_\_\_ Leaf Feeding  
       \_\_\_\_ Stalk Tunneling  
       \_\_\_\_ cm tunneled/plant
- 25     Two-spotted Spider Mite (*Tetranychus urticae*)  
     Western Rootworm (*Diabrotica virgifrea virgifrea*)  
     Other (Specify): \_\_\_\_\_

**30    12. AGRONOMIC TRAITS:**

- Staygreen (at 65 days after anthesis; rate from 1-worst to 9-excellent)  
% Dropped Ears (at 65 days after anthesis)  
% Pre-anthesis Brittle Snapping  
% Pre-anthesis Root Lodging  
35     % Post-anthesis Root Lodging (at 65 days after anthesis)  
% Post-anthesis Stalk Lodging  
7,891.0 Kg/ha (Yield at 12-13% grain moisture)

TABLE 2  
SSR PROFILE OF PHCWK

Bin #	Marker Name	Base Pairs
1.01	BNLG1014	116.16
1.01	UMC2215	144.91
1.02	BNLG1007	134.75
1.02	BNLG1083	221.66
1.02	BNLG1127	114.28
1.02	BNLG1953	248.02
1.03	BNGL439	232.18
1.03	PHI109275	132.76
1.04	BNLG1016	251.73
1.04	BNLG2086	225.05
1.05	BNLG1832	233.43
1.05	BNLG1886	147.73
1.06	BNLG1057	247.08
1.06	BNLG1615	219.23
1.07	BNLG1556	178.52
1.09	BNLG1331	119.4
1.09	BNLG1720	236.57
1.09	PHI011	212.86
1.09	UMC1306	134.6
1.1	PHI308707	117
1.11	PHI064	82.6
1.11	PHI227562	322.63
1.11	PHI265454	217.53
1.11	UMC1630	185.46
2.01	PHI96100	280.07
2.01	UMC1227	139.52
2.02	BNLG1017	195.39
2.02	UMC1265	302.3
2.03	BNLG1064	200.59
2.03	PHI109642	147.9
2.04	PHI083	129.02
2.04	UMC1326	142.96
2.05	UMC1275	314.26
2.06	BNLG1036	163.26
2.06	BNLG1138	218.13
2.06	BNLG1396	133.44
2.06	BNLG1831	190.13
2.07	UMC1560	152.99
2.08	BNLG1141	180.68
2.08	BNLG1258	177.15
2.08	BNLG1940	208.48

Bin #	Marker Name	Base Pairs
2.08	PHI435417	217.86
2.08	UMC1049	129.76
2.09	BNLG1520	285.99
2.09	UMC1736	284.61
2.1	PHI101049	226.4
3	PHI453121	211.71
3.01	PHI104127	169.51
3.01	PHI404206	298.76
3.02	BNLG1647	179.81
3.02	PHI243966	211.17
3.04	BNLG1452	131.5
3.04	BNLG1638	198.86
3.04	BNLG2241	142.46
3.05	BNLG1035	101.07
3.05	PHI073	185.61
3.06	BNLG1160	208.68
3.06	BNLG1951	124.93
3.06	PHI102228	135.66
3.09	BNLG1496	233.34
3.1	UMC1136	145.43
4	PHI072	139.92
4.01	PHI295450	197.06
4.03	ADH2	117.96
4.05	BNLG1159	145.81
4.05	BNLG1265	192.5
4.05	BNLG1755	240.32
4.05	BNLG1937	237.87
4.05	UMC1175	282.21
4.07	BNLG1784	229.46
4.07	UMC2038	135.48
4.11	BNLG1890	200.68
4.11	PHI076	170.08
5.03	PHI109188	161.35
5.04	BNGL653	151.47
5.04	BNLG1208	118.8
5.04	BNLG653	152.68
5.05	PHI333597	216.23
5.05	UMC2386	124.6
5.06	PHI085	256.45
5.07	BNLG1118	82.62
5.07	BNLG1346	175.42
5.07	BNLG1711	177.09
6.01	BNLG1422	215.98
6.04	UMC2317	129.67
6.05	BNLG1174	222.8

Bin #	Marker Name	Base Pairs
6.06	UMC1463	301.61
6.07	BNLG1740	236.52
6.07	PHI299852	119.8
7	BNLG2132	222.7
7.01	UMC1159	227.99
7.02	BNLG1094	153.5
7.02	PHI034	119.84
7.02	UMC2327	130.67
7.03	BNLG1070	145.52
7.03	BNLG2271	233.62
7.03	UMC1713	138.75
7.04	PHI328175	128.12
7.06	PHI116	169.95
8.01	UMC1075	260.75
8.03	BNLG1863	272.29
8.03	PHI100175	136.76
8.03	PHI115	303.03
8.03	PHI121	93.25
8.04	BNLG2046	320.53
8.05	BNLG1176	225.95
8.06	UMC1149	216.54
8.07	BNLG1065	237.06
8.08	BNLG1056	94.63
8.08	PHI015	82.71
8.09	PHI233376	135.77
8.09	UMC1663	201.45
9.01	BNLG1810	211.05
9.01	BNLG2122	221.36
9.01	PHI033	249.12
9.01	UMC1867	238.42
9.03	PHI079	187.4
9.04	BNLG1012	161.56
9.06	PHI448880	176.97
9.08	BNLG1129	290.49
10	PHI041	202.03
10.01	UMC1291	138.69
10.02	BNLG1429	205.1
10.02	PHI059	152.96
10.03	BNLG1079	172.81
10.03	UMC1037	228.71
10.04	PHI062	161.16
10.05	BNLG1074	184.18
10.07	BNLG1450	184.87

**TABLE 3A**  
**PAIRED INBRED COMPARISON REPORT**  
**Variety #1: PHCWK**  
**Variety #2: PH5TG**

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT ABS	EGRWTH SCORE ABS	ESTCNT COUNT ABS	TILLER PCT ABS
Mean1	125.7	133.7	14.7	5.6	22.9	3.7
Mean2	105.8	109.9	15.5	6.2	21.5	9.1
Locs	8	8	8	15	17	12
Reps	16	16	16	15	18	12
Diff	19.9	23.8	0.8	-0.6	1.4	5.3
Prob	0.046	0.047	0.521	0.023	0.243	0.390

Stat	GDUSHD GDU ABS	GDUSLK GDU ABS	POLWT VALUE ABS	POLWT VALUE %MN	TASBLS SCORE ABS	TASSZ SCORE ABS
Mean1	118.4	119.6	68.5	53.5	9.0	4.1
Mean2	121.9	126.1	103.7	81.5	9.0	4.4
Locs	38	38	15	15	2	32
Reps	38	38	30	30	3	32
Diff	-3.5	-6.5	-35.1	-28.1	0.0	-0.3
Prob	0.000	0.000	0.000	0.000	1.000	0.070

Stat	PLHTH CM ABS	EARHT CM ABS	STAGRN SCORE ABS	STKLDG %NOT ABS	SCTGRN SCORE ABS	EARMLD SCORE ABS
Mean1	223.4	92.2	3.7	65.0	8.5	7.3
Mean2	204.7	83.3	4.0	80.0	6.5	8.7
Locs	21	8	3	1	6	3
Reps	21	8	3	1	6	3
Diff	18.7	8.9	-0.3	-15.0	2.0	-1.3
Prob	0.000	0.074	0.742	.	0.041	0.270

Stat	BARPLT %NOT ABS	GLFSPT SCORE ABS	NLFBLT SCORE ABS	ANTROT SCORE ABS	FUSERS SCORE ABS	EYESPT SCORE ABS
Mean1	98.8	3.5	6.0	4.0	7.0	4.0
Mean2	95.3	4.5	7.0	8.0	8.8	5.5
Locs	14	1	1	1	4	1
Reps	14	2	1	2	4	2
Diff	3.5	-1.0	-1.0	-4.0	-1.8	-1.5
Prob	0.046	.	.	.	0.035	.

Stat	COMRST SCORE ABS	CLDTST PCT ABS	CLDTST PCT %MN	KSZDCD PCT ABS	HD SMT %NOT ABS	ERTLPN %NOT ABS
Mean1	7.0	90.0	98.2	3.4	100.0	100.0
Mean2	7.0	90.6	99.0	7.4	100.0	100.0
Locs	1	13	13	13	1	2
Reps	1	13	13	13	2	2
Diff	0.0	-0.6	-0.8	-4.0	0.0	0.0
Prob	.	0.680	0.653	0.001	.	1.000

Stat	LRTLPN %NOT ABS
Mean1	95.0
Mean2	95.0
Locs	2
Reps	2
Diff	0.0
Prob	1.000

TABLE 3B  
 PAIRED INBRED COMPARISON REPORT  
 Variety #1: PHCWK  
 Variety #2: PH1CN

Stat	EGRWTH SCORE ABS	ESTCNT COUNT ABS	TILLER PCT ABS	GDUSHD GDU ABS	GDUSLK GDU ABS
Mean1	6.5	22.0	1.1	120.1	120.4
Mean2	5.0	14.0	0.0	128.9	130.4
Locs	2	2	3	7	7
Reps	2	2	3	7	7
Diff	1.5	8.0	-1.1	-8.7	-10.0
Prob	0.205	0.500	0.423	0.009	0.003

Stat	TASBLS SCORE ABS	TASSZ SCORE ABS	PLTHT CM ABS	EARHT CM ABS	STAGRN SCORE ABS
Mean1	9.0	4.7	222.3	89.4	1.0
Mean2	9.0	5.0	204.6	81.9	2.0
Locs	2	7	5	2	1
Reps	2	7	5	2	1
Diff	0.0	-0.3	17.7	7.5	-1.0
Prob	1.000	0.172	0.009	0.500	.

Stat	BARPLT %NOT ABS	ERTLPN %NOT ABS	LRTLPN %NOT ABS
Mean1	100.0	100.0	70.0
Mean2	96.7	100.0	100.0
Locs	4	1	1
Reps	4	1	1
Diff	3.3	0.0	-30.0
Prob	0.234	.	.

TABLE 3C  
 PAIRED INBRED COMPARISON REPORT  
 Variety #1: PHCWK  
 Variety #2: PHAA0

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT ABS	EGRWTH SCORE ABS	ESTCNT COUNT ABS	TILLER PCT ABS
Mean1	125.7	133.7	14.7	5.6	22.9	3.7
Mean2	118.0	125.3	14.9	6.4	20.1	0.0
Locs	8	8	8	15	17	12
Reps	16	16	16	15	18	12
Diff	7.7	8.4	0.2	-0.8	2.9	-3.7
Prob	0.038	0.051	0.852	0.003	0.011	0.154

Stat	GDUSHD GDU ABS	GDUSLK GDU ABS	POLWT VALUE ABS	POLWT VALUE %MN	TASBLS SCORE ABS	TASSZ SCORE ABS
Mean1	118.4	119.6	74.8	58.9	9.0	4.1
Mean2	117.3	118.8	82.3	63.4	9.0	3.0
Locs	38	38	19	19	2	32
Reps	38	38	36	36	3	32
Diff	1.0	0.8	-7.5	-4.5	0.0	1.1
Prob	0.096	0.164	0.258	0.376	1.000	0.000

Stat	PLHTH CM ABS	EARHT CM ABS	STAGRN SCORE ABS	STKLDG %NOT ABS	SCTGRN SCORE ABS	EARMLD SCORE ABS
Mean1	223.4	92.2	3.7	65.0	8.5	7.3
Mean2	192.4	75.4	4.3	36.8	8.3	8.0
Locs	21	8	3	1	6	3
Reps	21	8	3	1	6	3
Diff	31.0	16.8	-0.7	28.2	0.2	-0.7
Prob	0.000	0.006	0.529	.	0.611	0.423

Stat	BARPLT %NOT ABS	GLFSPT SCORE ABS	NLFBLT SCORE ABS	ANTROT SCORE ABS	FUSERS SCORE ABS	EYESPT SCORE ABS
Mean1	98.8	3.5	6.0	4.0	7.0	4.0
Mean2	98.5	3.5	7.0	8.0	8.5	5.5
Locs	14	1	1	1	4	1
Reps	14	2	1	2	4	2
Diff	0.2	0.0	-1.0	-4.0	-1.5	-1.5
Prob	0.707	.	.	.	0.103	.

Stat	COMRST SCORE ABS	CLDTST PCT ABS	CLDTST PCT %MN	KSZDCD PCT ABS	HD SMT %NOT ABS	ERTLPN %NOT ABS
Mean1	7.0	89.2	97.9	2.8	100.0	100.0
Mean2	8.0	91.7	100.7	6.9	100.0	100.0
Locs	1	12	12	12	1	2
Reps	1	12	12	12	2	2
Diff	-1.0	-2.5	-2.8	-4.2	0.0	0.0
Prob	.	0.215	0.211	0.001	.	1.000

Stat	LRTLPN %NOT ABS
Mean1	95.0
Mean2	95.0
Locs	2
Reps	2
Diff	0.0
Prob	1.000

TABLE 3D  
PAIRED INBRED COMPARISON REPORT  
Variety #1: PHCWK  
Variety #2: PH75K

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT ABS	EGRWTH SCORE ABS	ESTCNT COUNT ABS	TILLER PCT ABS
Mean1	126.6	133.3	14.5	5.7	22.8	3.2
Mean2	102.5	106.8	14.4	6.1	21.8	1.2
Locs	4	4	4	17	18	15
Reps	8	8	8	17	18	15
Diff	24.0	26.5	0.0	-0.4	0.9	-2.0
Prob	0.012	0.019	0.921	0.110	0.456	0.386

Stat	GDUSHD GDU ABS	GDUSLK GDU ABS	TASBLS SCORE ABS	TASSZ SCORE ABS	PLTHT CM ABS	EARHT CM ABS
Mean1	118.6	119.8	9.0	4.2	223.2	91.6
Mean2	119.8	120.1	9.0	4.3	188.4	77.0
Locs	45	45	3	39	26	10
Reps	45	45	3	39	26	10
Diff	-1.2	-0.3	0.0	-0.1	34.8	14.7
Prob	0.028	0.505	1.000	0.584	0.000	0.010

Stat	STAGRN SCORE ABS	STKLDG %NOT ABS	SCTGRN SCORE ABS	EARMLD SCORE ABS	BARPLT %NOT ABS	GLFSPT SCORE ABS
Mean1	3.0	65.0	8.5	6.5	99.0	3.5
Mean2	3.3	85.0	7.0	7.5	94.1	3.0
Locs	4	1	6	2	18	1
Reps	4	1	6	2	18	2
Diff	-0.3	-20.0	1.5	-1.0	5.0	0.5
Prob	0.391	.	0.107	0.500	0.027	.

Stat	NLFBLT SCORE ABS	ANTROT SCORE ABS	FUSERS SCORE ABS	EYESPT SCORE ABS	COMRST SCORE ABS	CLDTST PCT ABS
Mean1	6.0	4.0	7.0	4.0	7.0	86.8
Mean2	7.0	8.5	7.8	5.5	8.0	92.8
Locs	1	1	4	1	1	4
Reps	1	2	4	2	1	4
Diff	-1.0	-4.5	-0.8	-1.5	-1.0	-6.0
Prob	.	.	0.215	.	.	0.078

Stat	CLDTST PCT %MN	KSZDCD PCT ABS	HD SMT %NOT ABS	ERTLPN %NOT ABS	LRTLPN %NOT ABS
Mean1	96.2	2.5	100.0	100.0	86.7
Mean2	102.9	4.3	100.0	95.3	73.3
Locs	4	4	1	3	3
Reps	4	4	2	3	3
Diff	-6.7	-1.8	0.0	4.7	13.3
Prob	0.082	0.188	.	0.423	0.732

TABLE 4  
GENERAL COMBINING ABILITY REPORT  
PHCWK

PRM Day ABS	Mean	92
PRM Day ABS	Reps	2969
PRMSHD Day ABS	Mean	91
PRMSHD Day ABS	Reps	2989
YIELD bu/a 56# ABS	Mean	162.7
YIELD bu/a 56# ABS	Reps	1467
YIELD bu/a 56# ABS	Years	3
YIELD bu/a 56# %MN	Mean	100.9
YIELD bu/a 56# %MN	Reps	1467
MST pct ABS	Mean	23
MST pct ABS	Reps	1477
MST pct %MN	Mean	99.9
MST pct %MN	Reps	1477
STLPCN %NOT %MN	Mean	93
STLPCN %NOT %MN	Reps	501
STLLPN %NOT %MN	Mean	89
STLLPN %NOT %MN	Reps	297
ERTLPN %NOT %MN	Mean	119
ERTLPN %NOT %MN	Reps	42
LRTLPN %NOT %MN	Mean	99
LRTLPN %NOT %MN	Reps	145
TSTWT lb/bu %MN	Mean	100.9
TSTWT lb/bu %MN	Reps	883
STKCNT count %MN	Mean	100
STKCNT count %MN	Reps	2569
PLTHT in %MN	Mean	102
PLTHT in %MN	Reps	641
EARHT in %MN	Mean	102
EARHT in %MN	Reps	638
BRTSTK %NOT %MN	Mean	104
BRTSTK %NOT %MN	Reps	188
GLFSPT score ABS	Mean	2
GLFSPT score ABS	Reps	6
STAGRN score ABS	Mean	5
STAGRN score ABS	Reps	510
HSKCVR score ABS	Mean	6
HSKCVR score ABS	Reps	105

**TABLE 5A**  
**INBREDS IN HYBRID COMBINATION REPORT**  
**Variety #1: HYBRID CONTAINING PHCWK**  
**Variety #2: 38R92**

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT %MN	EGRWTH SCORE %MN	ESTCNT COUNT %MN	GDUSHD GDU %MN
Mean1	164.4	100.3	98.2	95.7	89.5	98.5
Mean2	165.2	100.9	100.9	102.7	100.4	98.9
Locs	14	14	14	16	6	12
Reps	27	27	27	32	12	19
Diff	-0.8	-0.6	2.7	-7.0	-10.9	-0.3
Prob	0.709	0.644	0.031	0.239	0.030	0.553

Stat	GDUSLK GDU %MN	STKCNT COUNT %MN	PLTHT CM %MN	EARHT CM %MN	STAGRN SCORE %MN	TSTWT LB/BU ABS
Mean1	98.8	97.4	105.3	106.0	82.1	52.5
Mean2	99.1	100.3	96.2	99.7	104.5	51.4
Locs	6	40	8	8	7	10
Reps	8	78	15	15	13	20
Diff	-0.3	-2.8	9.1	6.3	-22.3	1.1
Prob	0.620	0.000	0.000	0.010	0.004	0.036

Stat	ECB1LF SCORE ABS	ECB2SC SCORE ABS	HSKCVR SCORE ABS	GIBROT SCORE ABS	BRTSTK %NOT ABS	LRTLPN %NOT ABS
Mean1	9.0	8.7	7.0	3.0	93.1	95.0
Mean2	6.0	4.7	4.5	2.0	86.4	100.0
Locs	1	3	1	1	4	1
Reps	2	6	2	2	8	2
Diff	3.0	4.0	2.5	1.0	6.7	-5.0
Prob	.	0.015	.	.	0.484	.

**TABLE 5B**  
**INBREDS IN HYBRID COMBINATION REPORT**  
**Variety #1: HYBRID CONTAINING PHCWK**  
**Variety #2: 38G60**

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT %MN	EGRWTH SCORE %MN	ESTCNT COUNT %MN	GDUSHD GDU %MN
Mean1	157.7	101.8	99.8	96.4	94.8	98.8
Mean2	156.4	100.5	97.0	86.6	109.9	100.2
Locs	36	36	36	10	14	27
Reps	74	74	75	20	31	48
Diff	1.3	1.2	-2.8	9.8	-15.1	-1.3
Prob	0.490	0.338	0.033	0.239	0.019	0.000

Stat	GDUSLK GDU %MN	STKCNT COUNT %MN	PLTHT CM %MN	EARHT CM %MN	STAGRN SCORE %MN	STKLDG %NOT %MN
Mean1	98.5	100.0	101.5	99.5	95.4	98.8
Mean2	101.0	101.3	102.0	98.5	105.3	100.1
Locs	14	69	15	15	15	2
Reps	19	135	32	32	32	4
Diff	-2.4	-1.3	-0.5	0.9	-9.9	-1.3
Prob	0.001	0.001	0.578	0.667	0.273	0.500

Stat	TSTWT LB/BU ABS	ANTROT SCORE ABS	FUSERS SCORE ABS	GIBERS SCORE ABS	ECB2SC SCORE ABS	HSKCVR SCORE ABS
Mean1	53.4	2.0	9.0	8.0	4.9	5.9
Mean2	51.6	5.5	9.0	7.5	3.5	5.9
Locs	24	1	1	2	4	8
Reps	50	2	2	4	10	16
Diff	1.8	-3.5	0.0	0.5	1.4	-0.1
Prob	0.000	.	.	0.500	0.325	0.882

Stat	GIBROT SCORE ABS	BRTSTK %NOT ABS	HD SMT %NOT ABS	ERTLPN %NOT ABS	LRTLPN %NOT ABS
Mean1	2.0	94.3	71.3	89.0	100.0
Mean2	3.0	83.3	98.3	85.4	90.3
Locs	1	5	3	5	3
Reps	2	10	4	10	5
Diff	-1.0	11.0	-27.0	3.6	9.7
Prob	.	0.260	0.209	0.681	0.237

TABLE 5C  
 INBREDS IN HYBRID COMBINATION REPORT  
 Variety #1: HYBRID CONTAINING PHCWK  
 Variety #2: 38R69

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT %MN	EGRWTH SCORE %MN	ESTCNT COUNT %MN	GDUSHD GDU %MN
Mean1	163.5	100.6	98.2	93.9	97.5	98.7
Mean2	160.1	98.5	100.0	104.7	96.0	98.5
Locs	48	48	48	10	12	27
Reps	72	72	72	18	25	40
Diff	3.4	2.2	1.8	-10.9	1.6	0.2
Prob	0.147	0.120	0.090	0.031	0.771	0.607

Stat	GDUSLK GDU %MN	STKCNT COUNT %MN	PLTHT CM %MN	EARHT CM %MN	STAGRN SCORE %MN	STKLDG %NOT %MN
Mean1	98.5	100.0	101.7	99.9	94.6	98.8
Mean2	97.6	99.1	94.7	100.0	107.2	100.1
Locs	14	79	15	15	15	2
Reps	19	131	25	25	27	4
Diff	0.9	0.8	7.0	-0.1	-12.5	-1.3
Prob	0.078	0.054	0.000	0.956	0.030	0.500

Stat	TSTWT LB/BU ABS	NLFBLT SCORE ABS	ANTROT SCORE ABS	FUSERS SCORE ABS	GIBERS SCORE ABS	ECB2SC SCORE ABS
Mean1	53.9	1.0	2.0	9.0	8.0	4.7
Mean2	52.6	2.5	2.5	8.0	6.0	3.8
Locs	19	2	1	1	2	8
Reps	33	2	2	2	4	14
Diff	1.3	-1.5	-0.5	1.0	2.0	0.9
Prob	0.000	0.205	.	.	0.410	0.471

Stat	HSKCVR SCORE ABS	GIBROT SCORE ABS	BRTSTK %NOT ABS	HD SMT %NOT ABS	ERTLPN %NOT ABS	LRTLPN %NOT ABS
Mean1	5.9	2.0	97.1	75.8	85.8	100.0
Mean2	4.2	1.0	87.1	95.4	55.3	99.2
Locs	7	1	4	4	6	3
Reps	14	2	8	5	11	5
Diff	1.6	1.0	9.9	-19.6	30.6	0.8
Prob	0.039	.	0.246	0.153	0.016	0.423

TABLE 5D  
 INBREDS IN HYBRID COMBINATION REPORT  
 Variety #1: HYBRID CONTAINING PHCWK  
 Variety #2: 38R92

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT %MN	EGRWTH SCORE %MN	ESTCNT COUNT %MN	GDUSHD GDU %MN
Mean1	158.9	100.8	98.4	98.6	94.1	98.8
Mean2	156.7	99.5	103.4	94.5	92.7	98.9
Locs	48	48	48	26	20	38
Reps	92	92	93	52	43	64
Diff	2.2	1.3	5.0	4.1	1.5	0.0
Prob	0.154	0.207	0.000	0.336	0.731	0.883

Stat	GDUSLK GDU %MN	STKCNT COUNT %MN	PLTHT CM %MN	EARHT CM %MN	STAGRN SCORE %MN	STKLKG %NOT %MN
Mean1	98.7	99.2	102.4	100.6	92.5	98.8
Mean2	99.0	99.2	94.8	96.8	112.9	100.3
Locs	20	101	22	22	22	2
Reps	27	190	44	44	44	4
Diff	-0.3	0.0	7.6	3.8	-20.4	-1.5
Prob	0.301	0.999	0.000	0.010	0.000	0.677

Stat	TSTWT LB/BU ABS	ANTROT SCORE ABS	FUSERS SCORE ABS	GIBERS SCORE ABS	ECB1LF SCORE ABS	ECB2SC SCORE ABS
Mean1	52.8	2.0	9.0	8.0	6.0	5.3
Mean2	52.2	3.5	9.0	7.5	6.0	4.0
Locs	33	1	1	2	1	7
Reps	66	2	2	4	2	16
Diff	0.7	-1.5	0.0	0.5	0.0	1.3
Prob	0.011	.	.	1.000	.	0.146

Stat	HSKCVR SCORE ABS	GIBROT SCORE ABS	BRTSTK %NOT ABS	HD SMT %NOT ABS	ERTLPN %NOT ABS	LRTLPN %NOT ABS
Mean1	6.0	3.0	95.7	71.3	89.0	98.8
Mean2	5.7	2.0	87.6	95.3	63.5	99.6
Locs	9	2	9	3	5	4
Reps	18	4	17	4	10	7
Diff	0.3	1.0	8.1	-24.0	25.5	-0.9
Prob	0.621	0.500	0.066	0.240	0.082	0.581

TABLE 5E  
 INBREDS IN HYBRID COMBINATION REPORT  
 Variety #1: HYBRID CONTAINING PHCWK  
 Variety #2: 39K40

Stat	YIELD BU/A 56# ABS	YIELD BU/A 56# %MN	MST PCT %MN	EGRWTH SCORE %MN	ESTCNT COUNT %MN	GDUSHD GDU %MN
Mean1	179.8	109.1	102.7	91.7	96.0	99.6
Mean2	157.5	94.7	97.4	92.5	105.7	100.3
Locs	19	19	19	4	11	17
Reps	28	28	28	7	21	24
Diff	22.3	14.4	-5.2	-0.8	-9.7	-0.8
Prob	0.000	0.000	0.023	0.912	0.093	0.127

Stat	GDUSLK GDU %MN	STKCNT COUNT %MN	PLTHT CM %MN	EARHT CM %MN	STAGRN SCORE %MN	TSTWT LB/BU ABS
Mean1	99.1	99.8	101.2	98.2	108.0	54.7
Mean2	100.2	101.0	98.5	97.4	95.7	53.8
Locs	10	35	8	8	10	13
Reps	13	49	13	13	15	21
Diff	-1.1	-1.3	2.7	0.8	12.4	0.9
Prob	0.120	0.176	0.097	0.785	0.276	0.137

Stat	ECB2SC SCORE ABS	HSKCVR SCORE ABS	BRTSTK %NOT ABS	ERTLPN %NOT ABS	LRTLPN %NOT ABS
Mean1	6.0	5.2	99.2	88.8	90.0
Mean2	3.3	5.5	100.0	50.8	90.0
Locs	2	5	2	4	3
Reps	5	8	3	8	3
Diff	2.7	-0.3	-0.8	38.0	0.0
Prob	0.458	0.529	0.500	0.159	1.000

TABLE 5F  
 INBREDS IN HYBRID COMBINATION REPORT  
 Variety #1: HYBRID CONTAINING PHCWK  
 Variety #2: 38G60

Stat	GDUSHD GDU %MN	STKCNT COUNT %MN	PLTHT CM %MN	EARHT CM %MN
Mean1	99.2	99.7	105.1	102.9
Mean2	100.2	100.7	107.3	99.9
Locs	1	7	1	1
Reps	2	15	2	2
Diff	-1.0	-1.0	-2.2	3.1
Prob	.	0.595	.	.

TABLE 5G  
 INBREDS IN HYBRID COMBINATION REPORT  
 Variety #1: HYBRID CONTAINING PHCWK  
 Variety #2: 38R69

Stat	GDUSHD GDU %MN	STKCNT COUNT %MN	PLTHHT CM %MN	EARHT CM %MN
Mean1	99.2	99.7	105.1	102.9
Mean2	101.1	96.4	92.7	101.9
Locs	1	7	1	1
Reps	1	14	2	2
Diff	-1.9	3.3	12.4	1.0
Prob	.	0.206	.	.

All publications, patents and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All such publications, patents and patent applications are incorporated by reference herein for the purpose cited to the same extent as if each was specifically and

5 individually indicated to be incorporated by reference herein.

The foregoing invention has been described in detail by way of illustration and example for purposes of clarity and understanding. As is readily apparent to one skilled in the art, the foregoing are only some of the methods and compositions that illustrate the embodiments of the foregoing invention. It will be apparent to those of  
10 ordinary skill in the art that variations, changes, modifications and alterations may be applied to the compositions and/or methods described herein without departing from the true spirit, concept and scope of the invention.